BACTERIAL POPULATIONS AND THEIR ACTIVITY IN THE BENGUELA UPWELLING SYSTEM

by

David Gordon Muir

Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy.

University of Cape Town

1986

CONTENTS

DECLARATION

This thesis presents the results of original work which I carried out in the Department of Microbiology, University of Cape Town, between 1983 and 1986. None of the work presented here has been submitted in part or in whole for any other degree.

Much of the work results from collaborative studies but unless otherwise stated, reports in this thesis pertain only to my own data. Section 2 was carried out in collaboration with Dr C.L. Davis, Mr P. Fielding, Dr L.J. Seiderer and Dr T. Probyn. Figure 3a has previously appeared in Davis (1985).

Section 3 was carried out in collaboration with Ms S.J. Painting using apparatus designed by her, and data relating to nutrients are here used with her permission, and appear without prejudice to their subsequent use by her.

Section 4 was carried out in collaboration with the Sea Fisheries Research Institute, Cape Town. Raw data relating to salininity, temperature, density and nutrient chemistry are supplied by them to all participating scientists.

Data relating to bacterial numbers and biomass in Sections 3 and 4 are the result of collaboration between myself and Ms S.J. Painting and Ms F. Verheye-Dua.

Except where otherwise acknowledged, none of the work contained in this thesis has yet been published.

ABSTRACT

An investigation of variability in hydrological and bacterial parameters at a fixed coastal station (Oudekraal, Cape Peninsula 33°59'S 17°21'E) showed that bacterial populations varied in numbers and biomass on both a short term (daily) and seasonal basis, in response to changes in hydrological conditions which were largely wind induced.

During summer, regular and persistent offshore winds induced upwelling events during which cold, nutrient rich South Atlantic Central Water was advected to the surface and exported offshore. This upwelled water had a low bacterial biomass (<15.0µg $C.l^{-1}$), but a high diversity of genera and physiological types. On advection to the surface, upwelled water was rapidly colonised by phytoplankton, and associated with these blooms, an increase in bacterial numbers and biomass occurred. As a result, water sampled during inter-upwelling periods had a high bacterial biomass (>30.0µg $C.l^{-1}$). The growth of phytoplankton rapidly depleted the nutrients in upwelled water, such that they approached limiting levels within 5 days, but as offshore winds regularly induced new upwellings, nutrient stocks were constantly renewed, which

maintained both phytoplankton and bacterial populations in a young physiological state. As a result, little difference in the structure or diversity of plateable bacterial populations derived from upwelling or downwelling waters could be discerned during summer.

During winter, offshore winds and upwelling events were rare. Bacterial populations associated with these upwelling events were low in biomass and similar in structure and diversity to populations derived from upwelling water in summer, but during the lengthy inter-upwelling periods occurring during winter, the bacterial population increased in biomass but decreased in diversity as the upwelling population, which was dominated by fermentative isolates (Vibrio, Enterobacteriaceae) was replaced by one dominated by oxidative isolates (Pseudomonas, Acinetobacter).

The close association between bacterial population development and phytoplankton bloom development was confirmed during a 43 day incubation of upwelled water. On enclosure, the nutrient rich water was rapidly colonised by an extensive phytoplankton bloom. Associated with this bloom, a rapid increase in both numbers and biomass of bacteria dependent on labile D.O.C. and P.O.C. occurred. During this population increase, the initial plateable population, which was diverse and

dominated by fermentative isolates (Vibrio, Enterobacteriaceae) was replaced by a population of low diversity, dominated by oxidative isolates (Pseudomonas, Acinetobacter) similar in structure to populations of winter downwelling water. This population showed a wide range of catabolic properties, and was highly active in taking up ^{14}C radiolabelled glucose, alanine, aspartic acid and glutamic acid.

Following the development of the phytoplankton bloom, nutrient levels (NO_3) fell to very low levels (1µg $at.l^{-1}$) and the bloom subsequently senesced. Following this senescence, bacterial numbers and biomass fell to low levels, accompanied by a increase in diversity and the reappear- ance of fermentative isolates. Subsequent to the decline of this initial population, a second increase in the bacterial population occurred, producing high biomass levels (>200.0µg $C.l^{-1}$) of a population with low diversity, again dominated by oxidative isolates. This population, however, displayed a restricted range of catabolic properties, and failed to take up ^{14}C radiolabelled glucose, alanine, aspartic acid or glutamic acid, suggesting that it was dependent on recalcitrant D.O.C. and P.O.C. remaining from the initial phytoplankton bloom.

Transects of the Benguela region, from the shore to the oceanic front, both off the Cape Peninsula and off Cape Columbine, showed that highest bacterial biomass occurred in the euphotic zone close inshore, in association with waters rich in Chl a. The development of phytoplantkon blooms was strongly related to the presence of nutrient rich upwelled water, while the presence of fronts appeared to effect vertical mixing in the water column which maintained nutrients above limiting levels offshore. These transects confirmed the hypothesis that the biological characteristics of the surface upwelled waters was dependent on the length of time of exposure in the euphotic zone, and suggested that the oceanic front is characterised by the juxtaposition of a highly oligotrophic oceanic population with a more eutrophically adapted inshore population.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, Professor Frank Robb, for accepting me as a student and giving me encouragement and support throughout the project. My colleagues Dr Claire Davis, Dr Muffy Seiderer, Dr Trevor Probyn, Dr Mike Lucas, Mr Pete Fielding, Ms S. J. Painting and Ms Frieda Verheye-Dua were all involved in co-operative studies with me, and provided much support and incentive both practically and in discussion. I am particularly grateful to Sue Painting and Frieda Verheye-Dua, for their collaboration in the computation of A.O.D.C. and biomass estimates. I also thank Mr Ian Humphries, who counted many of the A.O.D.C. samples for us.

In addition, I owe an enormous debt of gratitude to members of the Sea Fisheries Research Institute, Cape Town. All cruises (Section 4) were organised by them, and in this regard I must particularly thank Dr Larry Hutchings, Ms Dot Armstrong and Ms Penny Brown who organised both frontal cruises. Thanks are also due to the technical staff of the S.F.R.I., who provided the hydrological and nutrient data, and to the captain and crew of the R.S. Africana.

Friends and family have provided enormous support to me

during this time; in particular I must thank my parents and sister Mary Smorenburg, Dr Klaus Koop and Dr Annie Linley, as well as all other friends.

I am grateful to Ms Genevieve Wilson and Jane Puhar and other personnel of General Business Systems (WANG) who typed this thesis.

This work was supported by funds from the Benguela Ecology Program of the South African National Committee for Oceanographic Research.

SECTION 1: GENERAL INTRODUCTION

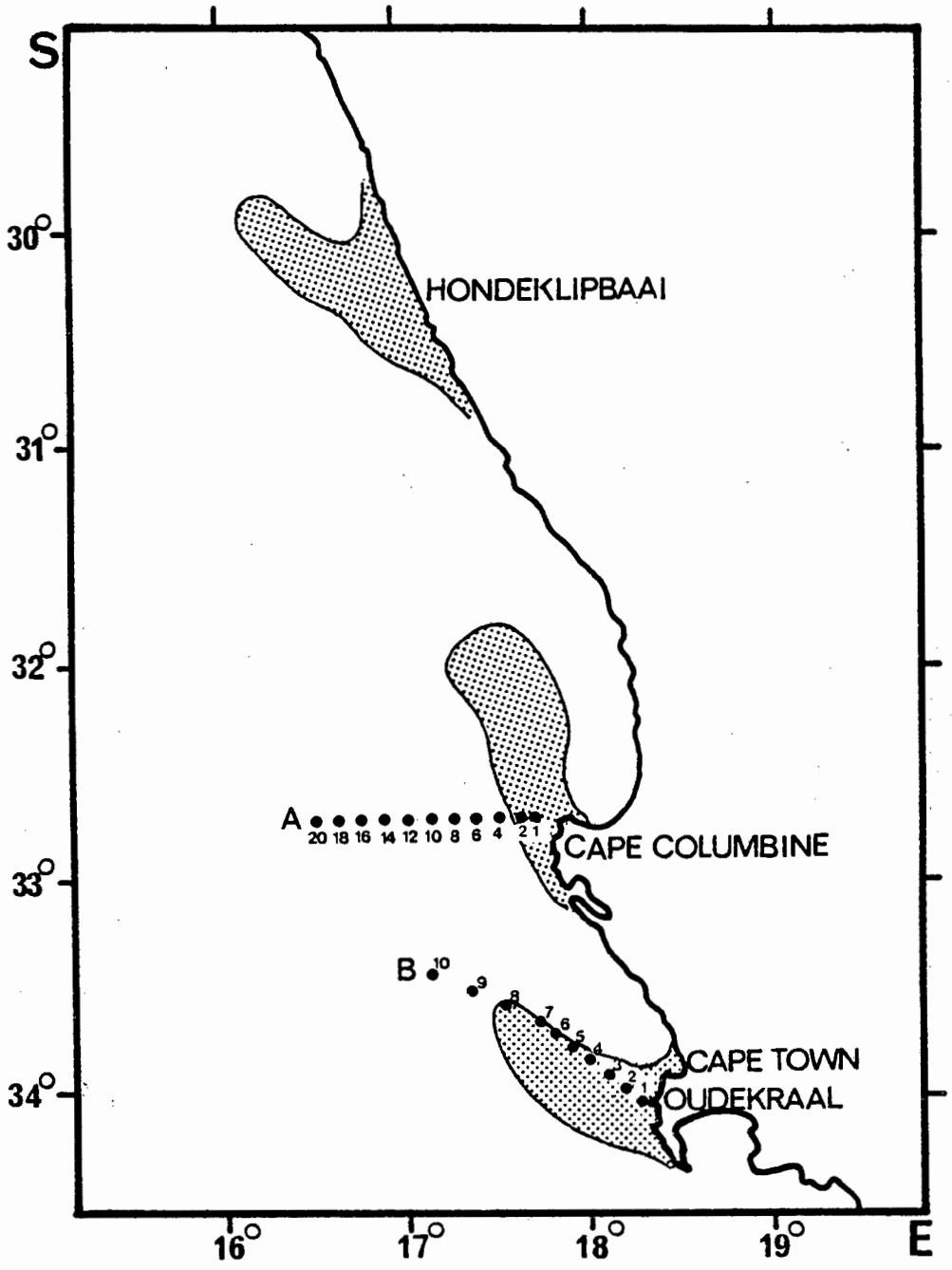

FIGURE 1: Map of the southern portion of the Benguela Upwelling Region, showing 3 prominent upwelling sites (▨). Line A shows the 20 stations comprising the Cape Columbine frontal zone transect (Section 4.3). Line B shows the 10 stations comprising the Upwelling Monitoring Line (Section 4.2).

INTRODUCTION

Marine microbial communities are complex assemblages of a variety of prokaryotes and eukaryotes of varying size, morphology and physiological state (Lucas, 1985). The heterotrophic populations in particular are genetically diverse, although dominated by gram-negative cells, and display a wide range of physiological properties.

Heterotrophic bacteria have a number of characteristics which confer on them a significant strategic advantage in an oligotrophic environment where sources of nutrient may be transitory and rare: they can utilise dissolved organic and inorganic nutrient at very low substrate concentrations, and many have been shown to have uptake mechanisms specific to rare substrates (Morita, 1984a; Button, 1985; Davis and Robb, 1985). They show a wide range of catabolic properties and are able to use highly refractory materials such as plant polymers (Fenchel and Jorgensen, 1977; Fenchel and Blackburn, 1979) and in addition are small and have a high surface-volume ratio - Williams (1981) showed that although bacterial biomass in a CEPEX enclosure was only 4.6% of the total planktonic biomass, bacterial surface area was 69% of the total of all organisms. Furthermore, bacteria have a short lag phase and rapid doubling rate, and are thus able to take quick advantage of favourable conditions (Pomeroy, 1984). In addition,

many marine heterotrophs exhibit starvation-survival adaptions which enable them to survive long periods of unfavourable conditions (Novitsky and Morita, 1976, 1978; Tabor et al., 1981; Torrella and Morita, 1982; Amy and Morita, 1983a, b; Morita, 1982).

Even in a diverse range of marine habitats such as tropical, temperate, arctic, nearshore and oceanic water masses, bacterial numbers are fairly high and consistent ($1 \times 10^5 - 1 \times 10^6$ cells.ml^{-1} van Es and Meyer-Reil, 1982; Azam et al., 1982) and most appear to be free living (Pomeroy, 1984; Lucas, 1985). Estimates of bacterial biomass vary widely, however, from 1 to 200µg C.l^{-1}, and appear to be closely linked to the availability of nutrient, and in particular carbon, in the system (Meyer-Reil, 1977; van Es and Meyer-Reil, 1982; Wright, 1984; Wangersky, 1984).

A close association between bacterial biomass and activity and primary production has been demonstrated for both fresh and marine waters. Both plankton and macro-algae secrete a variety of compounds as dissolved organic matter (D.O.M.). The rate of release varies with physiological state, but may represent up to 50% of photosynthetically derived organic carbon (P.D.O.C.) (Ignatiades and Fogg, 1973; Williams 1981a; Larsson and Hagstrom, 1979, 1982; Lancelot, 1984). P.D.O.C. consists of a wide variety of

compounds such as glucose (Hellebust, 1970; Eberlein et al., 1983), mannitol (Newell et al., 1980) and other carbohydrates as well as amino acids (Crawford et al. 1974; Bright and Fletcher, 1983a, b) and vitamins (Lancelot, 1984). Between 9-48% of P.D.O.C. consists of small molecules (<500d) which are rapidly taken up and utilised by bacteria, whilst more refractory substances requiring modification by extracellular enzyme hydrolysis are taken up more slowly. Up to 90% of P.D.O.C. production is utilised by bacteria at rates of between 5-40%.h^{-1} (Derenbach and Williams, 1974; Lancelot, 1979; Larsson and Hagstrom, 1979, 1982; Wolter, 1982; Lancelot, 1984; Lucas, 1985) and can meet between 60% and 100% of heterotrophic bacterial requirements (Joiris et al., 1982; Larsson and Hagstrom, 1982).

Not all P.D.O.C. is readily utilisable, however, and that proportion remaining after initial bacterial utilisation may form the basis of residual pools of D.O.C. in marine waters which vary between 0.3 and 1.2mg $C.l^{-1}$ (Strickland and Parsons, 1968; Morita, 1982, 1984b).

The utilisation of D.O.M. can be viewed as a temporal sequence in which small (<500d) molecules are used first and the larger and more refractory compounds later - this sequence may be accompanied by successions in the

heterotrophic community (Jones, 1977; Martin, 1980). In investigations into the heterotrophic utilisation of kelp exudates, Linley et al. (1981) showed that successions of bacterial morphotypes occurred, and Martin (1980) and Martin and Bianchi (1980) showed similar changes in association with natural phytoplankton blooms.

There is also an association between elevated levels of particulate organic matter (P.O.M.) and increased abundance of bacteria (Goulder, 1977; Hanson and Wiebe, 1977; Itturiaga, 1979; Wangersky, 1984). Physical damage, fragmentation, grazing and senescence of planktonic and macro-algae all result in the formation of complex particulates. In addition, less than 10% of macro-algal production is consumed directly by herbivores (Field, 1984; Lucas, 1985) and much of the macro-algal production enters the detrital pool. 70% of the annual production of Ecklonia maxima and Laminaria pallida kelp on the west coast of South Africa enters the water column as P.O.M. and wrack (Newell and Lucas, 1981). Of that proportion cast up on the beach, 74% was consumed by amphipods and dipteran larvae, as shown in studies by Griffiths and Stenton-Dozey (1981), Koop and Lucas (1983) and Koop et al. (1982a, b), but as these herbivores had low assimilation efficiencies, 80% of this material was returned to the system in the form of faeces which were

utilised by bacteria. The importance of bacteria as mineralisers in this system has been detailed by studies on the heterotrophic utilisation of kelp and phytoplankton detritus and kelp exudates (Lucas et al., 1981; Linley et al., 1983; Newell et al., 1981; Stuart et al., 1981a).

In pelagic systems, phytoplankton dominates the primary productivity. Although in temperate areas between 50% and 90% of phytoplankton biomass is consumed by zooplankton (Field, 1984; Lucas, 1985), in upwelling systems the bloom and decay of phytoplankton takes place rapidly (Barlow, 1982b, 1984) and zooplankton turnover rates are too slow to produce populations capable of grazing more than about 3% of the phytoplankton biomass (Barlow, 1982b; Moloney et al., 1986). As a result, much of the phytoplankton biomass enters the detrital pool, particularly under conditions of nutrient limitation when blooms senesce (Barlow, 1984). Such detritus is rapidly and efficiently utilised by bacteria (Newell et al., 1981; Stuart et al., 1981a, b).

As a result of this close coupling between primary productivity and bacterial biomass, activity and productivity, areas of high primary productivity such as estuaries and inshore areas tend to have a high associated bacterial biomass. Since primary productivity is limited

principally by light and nutrients, areas of elevated primary productivity are limited to the euphotic zone of areas where nutrients are readily available.

Upwelling areas are characterised by the regular advection to the surface of nutrient rich bottom water, and are amongst the most productive areas of the world's oceans (Anciete, 1981). The hydrology of the inshore waters of the west coast of South Africa is dominated by such a system, which can be regarded, together with those of Peru, California and North-West Africa, as one of the major upwelling systems in the world's oceans. This upwelling is particularly intense at a few sites (fig.1) and occurs in response to south-easterly offshore winds which blow throughout the year, although they are most common in summer (Andrews and Hutchings, 1980; Jury et al., 1985a, b; Chapman and Shannon, 1985; Shannon, 1985b).

Upwelled water is rich in nutrients such as NO_3, NO_2, PO_4 and SiO_3, but has a low phytoplankton population, since it originates far below the euphotic zone (Andrews and Hutchings, 1980). Although upwelled water initially carries a low D.O.C. and P.O.C. load, by the time it has passed through the inshore area, it may have acquired a substantial D.O.C. and P.O.C. concentration derived from the inshore macrophyte beds, together with the attendant

microflora. Koop et al. (1982a) have shown that mannitol fermenting bacteria specific to the nearshore region of the kelp beds may be exported for some distance offshore during upwelling.

Once advected to the surface, upwelled water forms plumes and patches which move offshore (Hutchings, 1981; Hutchings et al., 1984). The extent to which they are maintained, and the distance that they move, is largely determined by the wind history, but Hutchings (1981) suggests that they may be maintained for >5 days before being dispersed by mixing or returned to the shore by wind reversals (downwelling).

Once upwelled water has reached the euphotic zone it is subject to rapid phytoplankton blooms, which may take only 5 days to reach late log phase (Barlow, 1982a, b). This growth depletes the nutrients and the bloom then enters a senescent phase (Barlow, 1984). The rapidity with which the bloom develops is dependant on the hydrological conditions, the nutrient concentration of the water and on the size of the seeding population (Brown and Field, 1986).

To date, no work has been done on the development of bacterial populations associated with the development of phytoplankton blooms in the Benguela system. Extensive work on the degradation of kelp detritus (Newell et al., 1980; Linley and Newell, 1981; Stuart et al., 1981a, b)

kelp exudates (Linley et al., 1981; Lucas et al., 1981) and phytoplankton detritus (Newell et al., 1981) have demonstrated the central role which heterotrophic bacteria play in nutrient cycling, but all of these studies have regarded the bacterial community as being homogenous in structure and activity. Martin (1980), Martin and Bianchi (1980) and Fukami et al., (1985a, b) on the other hand, have demonstrated that significant changes in the structure and activity of bacterial communities takes place in association with different phases of phytoplankton bloom development and decay. Mazure (1977, 1978) and Davis (1985) have shown that the heterotrophic population of the kelp beds is composed of a variety of taxonomic groups such as Vibrio, Pseudomonas, Acinetobacter, Flavobacterium and members of the Enterobacteriaceae, and that the numbers, biomass and structures of the population varies seasonally. Mazure (1978) hypothesised that seasonal rates of primary production and detritus formation influenced the structure of the bacterial population, but sampling in that study was conducted monthly, and given the rapid development of phytoplankton blooms in the Benguela upwelling system, it is unlikely that short term variability would be shown.

Davis et al. (1983) showed that discrete assemblages of bacteria were maintained in 4 closely related habitats in the Benguela system (beached kelp, interstitial water,

inshore water, offshore water) and suggested that significantly different populations of bacteria arose and were maintained by specific genetic traits which conferred a selective advantage in those habitats (Robb et al., 1985). Given the short lag time and rapid turnover rate of bacteria, it is probable that rapid changes in structure and activity of heterotrophic populations arise in the Benguela system in response to short-term variations in hydrological conditions, but no studies have been conducted to date which describe this.

Several problems attach to any such study of natural microbial populations. Without recourse to such techniques as immunofluorescent labelling (Dahle and Laake, 1982; Laake et al., 1983b), any study attempting to follow changes in the structure of the bacterial population has to utilise bacteria isolated in some way - this is usually done on solid media. It has been widely observed, however, that the viable count on solid media is usually very much lower than the total count observed in natural waters (Buck, 1979), an effect largely ascribed to the adaption of much of the population to oligotrophic conditions (Morita, 1984) and to the selective nature of most media.

Populations of bacteria in upwelling water have been subjected to starvation conditions for a long time.

The bottom residence time of South Atlantic Central Water is very long (>500 yrs) (Morita, 1984b; Shannon, 1985b) and has a low organic carbon content. As shown by Novitsky and Morita (1976, 1977, 1978), Tabor et al. (1981), Torrella and Morita (1982), Morita (1982), Amy and Morita (1983a, b), Kurath and Morita (1983) and Morita (1984a, b), marine bacteria such as Vibrio and Pseudomonas show specific starvation-survival adaptations which enable them to enter into a state of dormancy to wait out long periods of highly oligotrophic conditions. This enables the preservation of the genome at minimal metabolic expense (Sussman and Halvorsen, 1966) and such populations have low endogenous respiration rates and very low viability on normal solid media. As a result, viable counts of marine bacterial populations are often very low, and usually of the order of only 0.01 - 1.0% of the total count, since plating on solid media is in any event selective even amongst the active population (Buck, 1979).

Novitsky and Morita (1976) showed that a marine Vibrio (ANT 300) decreased in size and changed morphology from a rod to a small coccobacillus upon starvation. This was accompanied by an initial increase in viable count which was attributed to a fragmentation response. The endogenous respiration rate fell to 1% of its normal level, and the viability subsequently decreased markedly. Amy and Morita

(1983a, b) have found that the starvation survival response is widespread amongst marine genera, and Morita (1984a) has shown that despite their evident dormancy, high affinity uptake mechanisms are maintained in these cells which enable them to rapidly respond to the presence of very low levels of nutrient. Davis and Robb (1985) have shown that strains isolated from the Benguela Upwelling System (Vibrio, Pseudomonas) exhibited similar starvation-survival responses, and decreased in size and viability under starvation conditions, while maintaining high affinity uptake mechanisms. It has been suggested that the maintenance of uptake mechanisms may be the cause of low plateability of starved cells: although starved cells are able to take up substrates, they lack the ability to utilise them efficiently, being particularly deficient in RNA and mechanisms to neutralise free radical damage (Amy et al., 1983) - if suddenly subjected to high levels of nutrient, starved cells may start to revive, but die soon thereafter. MacDonell and Hood (1982) showed that small, starved cells required a slow increment of nutrient before they formed viable cells.

In the work described here, the structure and activity of heterotrophic bacterial communities of the Benguela Upwelling Region was examined in the area bounded by the coast and the oceanic front (fig.1). The structure of the

populations was assessed in terms of numbers, biomass and physiological and taxonomic groupings determined by numerical classification, while activity was determined using radioisotope techniques and determinations of levels of plateability. Parameters of bacterial population structure and activity were related to hydrological conditions, and the variability of the heterotrophic population with respect to short term and seasonal changes, and spatial changes with distance from the shore and depth were also examined. It was hypothesised that the major source of carbon in the system is derived from phytoplankton, and that hydrological and seasonal effects on the bacterioplankton are exerted principally through their effect on the phytoplankton.

Section 2 examines short term and seasonal variations in the structure of the bacterial population at a fixed sampling station on the coast in an upwelling zone. Changes in the bacterial population are related to changes in hydrological conditions. Section 3 examines the development of a heterotrophic bacterial community from newly upwelled water, associated with the development and decline of a phytoplankton bloom. Section 4 examines the structure and activity of bacterial communities in transects across the Benguela region from the

nearshore to the oceanic front, with special reference to the effect of frontal structures on the structure and activity of these populations.

SECTION 2: DAILY AND SEASONAL VARIABILITY IN HYDROLOGICAL AND BACTERIOLOGICAL PARAMETERS OF SEAWATER SAMPLED AT OUDEKRAAL, CAPE PENINSULA, SOUTH AFRICA (33°59'S, 17°21'E)

2.1 INTRODUCTION

Estimates of bacterial numbers and biomass in the sea vary considerably, depending largely on the origin of the sampled water. For example, Meyer-Reil (1982) and Van Es and Meyer-Reil (1982) cite numbers between 10 - 50 x 10^6 cells.mℓ$^{-1}$ for coastal waters, 0.5 - 10 x 10^6 cells.mℓ$^{-1}$ for offshore waters and 1.0 x 10^5 cells.mℓ$^{-1}$ for deep waters, while the biomass in coastal waters is estimated to range between 5 - 200µg C.l^{-1} and those of offshore waters between 1 - 5µg C.l^{-1} (Azam et al., 1983).

While such broad estimates of numbers and biomass offer an idea of the relative abundance of bacteria, they take no account of variability in the size of the bacterial population either on a short-term (for instance, daily) or long term (seasonal) basis, nor do they take into account spatial or habitat variations. In the Benguela system, for example, Davis et al. (1983) have shown that within 4 closely associated regions, distinct populations of bacteria occurred which varied in numbers, biomass and physiological response: the populations were maintained as discrete assemblages by their unique and characteristic responses to environmental conditions (Davis and Robb, 1985; Robb et al., 1985). In other systems, variability has been shown in bacterial communities both

spatially (for example, in vertical profiles [Sorokin, 1971; Gocke, 1977]) and temporally. Seasonal variations in particular are well documented: Sieburth (1967) showed that seasonal selection of bacterial populations in Narrangasett Bay occurred in response to environmental temperature changes, and similar systems have been described by Trentham and James (1981) and Murray and Hodson (1985) who showed that both temperature and levels of primary productivity caused changes in the spatial and seasonal distribution of bacterial biomass and activity in freshwater systems. Similarly Tabor and Neihof (1984) found variations in the number of metabolically active bacteria in Chesapeake Bay which were seasonally determined, while Palumbo et al. (1983) found variations in the uptake of amino acids which were determined by depth and season in the Newport River Estuary. Gocke (1977) showed marked horizontal, vertical and seasonal variations in the uptake rates of radiolabelled substrates in the Kiel Bight, which appeared to be strongly associated with changes in environmental nutrient levels, which in turn were related to levels of primary production (Bolter et al., 1977).

The association between bacteria and phytoplankton is well documented (Cole, 1982; Cole et al., 1982; Laake et al. 1983a, b; Lancelot and Billen, 1984). Bacterial numbers and biomass have been shown to be closely

correlated with Chl a levels (and hence phytoplankton biomass) in both freshwater and marine systems (Linley et al., 1983; Bird and Kalff, 1984; Painting et al., 1985) and the relationship seems to depend firstly upon the release by phytoplankton of dissolved exudates which are utilised as a nutritional resource by bacteria (Thomas, 1971; Cole, 1982; Cole et al., 1982; Eberlein et al., 1983) and secondly on the mineralisation of senescent or dead phytoplankton cells (Fenchel and Blackburn, 1979; Newell et al., 1981; Newell and Lucas, 1981; Linley et al., 1983; Newell et al., 1983; Fukami et al., 1985a, b). As a result of this close association, numbers and biomass of bacteria can reasonably be expected to vary with the physiological state of phytoplankton populations (Martin, 1980). In the Benguela region, it has been shown that the major areas of phytoplankton standing stocks and production lie close inshore, where they are associated with recently upwelled, nutrient rich waters (Brown, 1982, 1984; Brown and Hutchings, 1985; Shannon, 1984, 1985a, b). As a result of the episodic nature of upwelling, however, (Andrews and Hutchings, 1980; Jury et al., 1985a, b; Taunton-Clark, 1985) there is variability in the spatial and temporal distribution of phytoplankton activity on both a short and long term basis, which may occasion a similar variability in the associated bacterial populations. In early work on the

bacterial populations of the Benguela system, Mazure (1977, 1978, 1980) showed that seasonal changes in both bacterial numbers and biomass occurred, with maxima being recorded during the summer upwelling season (December, January, February) and minima during the winter months (June, July). These data were found to correlate well with phytoplankton standing stock estimates (Mazure, 1978). Barlow (1982a, b) however, shows that phytoplankton bloom development in newly upwelled water takes place in a matter of days, suggesting that within the longer term seasonal variation, significant short term variations may also occur in the bacterial population. Barlow (1984) describes bloom development in terms of lag, mature and senescent stages, which depend largely on the nutrient concentration, temperature and light availability. Martin (1980) and Martin and Bianchi (1980) show that different bacterial populations are associated with such stages of phytoplankton bloom development, whose taxonomic structure and physiological attributes are determined by the physiological state of the phytoplankton bloom.

Upwelling systems appear to be unique in the rapidity with which such phytoplankton blooms develop. In temperate waters the spring and autumn blooms may take weeks or months to develop (Morris and Skea, 1978), but in the Benguela system, blooms consistently develop

within 3 - 5 days after an upwelling event (Barlow, 1982b, 1984). While studies on temperate systems reveal that slow phytoplankton bloom developments are associated with changes in the bacterial populations (Delattre et al., 1979), little information is available on the short term changes associated with rapid changes in hydrological conditions and phytoplankton blooms in upwelling systems.

To monitor such changes, this work has used bacteria isolated on solid media. There is, however, some controversy over the relationship of plateable bacteria to the population as a whole. Plateable forms have been classified as saprophytic bacteria (Kuznetsov et al., 1979) to distinguish them from those which are only capable of growth at natural concentrations of organic matter ($< 5mg.l^{-1}$) but there have also been suggestions that plateable bacteria form a significant proportion of the active population (Bolter, 1977) and that plate counts form a sensitive indicator of the overall nutritional capability of the population (Laake et al., 1983b).

Levels of heterotrophic uptake ability in natural marine bacterial populations are variable - assessed by micro-autoradiographic methods using ^{3}H radiolabelled cells, Meyer-Reil (1978b) showed that an average of only 31.3% of bacteria in the Kiel Bight were metabolically active,

while Tabor and Neihof (1984), using a variety of methods, showed that between 25% and 85% of the population in Chesapeake Bay were metabolically active. Levels of activity are influenced by temperature and concentrations of nutrient (Gocke, 1977) and in general, decrease with depth and with distance offshore (Fuhrman et al., 1980; Novitsky, 1983), but although it has been suggested that organotrophic bacteria represent a major proportion of the active bacteria (Bolter, 1977), the C.F.U. count is usually lower than the number of actively metabolising cells (Gocke, 1977). Martin and MacLeod (1984) suggest that the distinction between organotrophic and eutrophic bacteria is spurious, and that the response of strains to increased nutrient is as dependent on the nature of the available nutrient as it is on the genetic characteristics of the organisms. MacDonell and Hood (1982), for example, showed that small cells resulting from starvation, revived by a gradual increment of nutrient, could be assigned to the same taxonomic groups as organotrophs in the same population.

The extent to which organotrophs are representative of the structure of the active population is thus difficult to determine, but Laake et al. (1983b), examining generic distributions in an enclosure, showed an excellent correlation between determinations based on immunofluorescent

techniques and standard plate assays. This suggests that the composition of the plateable population may be a useful indicator of the overall structure of the population, although it has to be borne in mind that plating is inevitably selective (Kuznetsov et al., 1979).

The characterisation of the plateable population must be based on a randomly drawn sample - while strain collections based initially on such characteristics as colony morphology provide information on the range of properties present in the population, they do not describe the frequency of occurrence of those properties. The number of isolates which should be selected, and the number of discriminatory tests to which they should be subjected, is also a matter of contention. Griffiths and Lovitt (1980) propose a method of numerical profiling with a restricted number of tests (9-12) in order that a large number of isolates can be rapidly screened, but other authors have subjected strains to >100 tests (reviewed in Atlas, 1984), although this reduces the number of isolates which can be tested. Bianchi and Bianchi (1982) suggest however that 20-30 strains subjected to about 30 discriminatory tests were sufficient to construct statistically valid indices of diversity in marine isolates.

The identification of marine bacteria, particularly to specific level, is difficult and taxonomic systems are in many ways inadequate to do so (Starr and Schmidt, 1982; Oliver, 1982; Atlas, 1984). Some system of grouping isolates on the basis of similarity is however essential if estimates of diversity are to be made and changes in population structure are to be followed. Diversity indices seek to describe the uncertainty that an individual picked at random will belong to a particular group within the community, and vary from simple indices such as the ratio of the number of groups to the number of individuals to more complex indices such as the Shannon-Weaver index (Shannon and Weaver, 1963) which incorporate functions to describe the distribution of characteristics within the population (Pielou, 1966; Bianchi and Bianchi, 1982; Legendre and Legendre, 1982; Atlas, 1984).

Most indices of diversity however have the disadvantage that they are sensitive to the size of the sample population and it is difficult to compare samples of widely differing sizes (Mills and Wassel, 1980). While statistical manipulation of the data by such techniques as the rarefaction method (Simberloff, 1978) can obviate this, the results of Bianchi and Bianchi (1982) suggest that within the limits of a sample size of 30-60 isolates, differences in sample size have little effect on the

construction of such indices as the Shannon-Weaver index. Indices of diversity based on information theory, such as the Troussellier and Legendre functional evenness index (Troussellier and Legendre, 1981) are independent of sample size and examine the evenness of distribution of properties in the sample, and are useful in comparing samples of different sizes but have the disadvantage that they do not reveal potentially significant groupings.

In the light of the difficulty of adequately identifying isolates, the techniques of numerical classification are commonly used to define groupings in bacterial assemblages. Such groups are defined by an index of similarity such as the Bray-Curtis measure (Clifford and Stephenson, 1975) based on a range of discriminatory tests, which need not necessarily be of taxonomic significance (Field et al., 1982).

Techniques of numerical classification have been used to describe specific groupings within the Vibrionaceae (Kaper et al., 1983) and Pseudomonodaceae (Colwell and Liston, 1961), to show dominance and diversity in natural phytoplankton stocks (Kaneta et al., 1985) to display habitat segregation in the Vibrionaceae (Simudu and Tsukamoto, 1985) and to follow seasonal and habitat changes in the saprophytic bacteria of the Kiel Bight

(Bolter, 1977). They have also been used to describe the fauna of South Africa benthic sediments and rocky shores (Field and McFarlane, 1968; Field, 1970, 1971) and to describe the habitat segregation of bacterial communities in the Benguela system (Davis et al., 1983).

Upwelling on the west coast of the Cape Peninsula, South Africa, is easily monitored by an examination of the water temperature and nutrient status from shore based stations. In this study, changes in hydrological conditions were monitored daily for 54 days during the summer upwelling season (29th October - 21st December) and for 31 days during winter (17th June - 17th July). Temperature and nutrient characteristics of the water were used to assess the hydrological status, and bacterial samples were assessed for total count, total biomass and plateability. For each of 9 selected days during summer and 10 selected days during winter, the taxonomic and physiological characteristics of the plateable population was determined.

2.2 Methods and Materials

2.2.1 Sampling and sampling site

All samples were taken at Oudekraal, Cape Peninsula,

South Africa (33°59'S 18°21'E) at 07h00 each day for 54 days during summer and for 31 days during winter. Samples were taken in about 3m of water from the surface, using sterile screw-capped 1ℓ bottles which were immediately transported to the laboratory. Duplicate 300mℓ subsamples were taken in dark bottles and pre-filtered through Whatman GF/F filters prior to being frozen (-4°C) for nutrient analysis. 10mℓ samples were preserved with Analar glutaraldehyde (2.0% final conc.) and stored at +4.0°C for acridine orange direct counting (A.O.D.C.) to determine the total count. Water temperature was determined with a standard laboratory thermometer, and wind direction and speed was estimated and noted at each sampling.

2.2.2 Nutrient analysis

Determinations of NO_3, NO_2, NH_4^+ and urea concentrations were performed on 5mℓ samples of water.

2.2.2.1 Nitrite and nitrate

NO_2 concentrations were determined using the methods of Nydahl (1976), in which NO_2 was reacted with sulphanilamide, which forms a diazonium salt which was then coupled to N-(1-naphthyl)ethylenediamine, forming a coloured aminoazo compound, whose concentration was

determined spectrophotometrically on a Pye-Unicam spectrophotometer with a path length of 1cm, at 543nm.

NO_3 concentrations were similarly determined, following the reduction of NO_3 to NO_2 in a cadmium reduction column, as described by Nydahl (1976).

2.2.2.2 Ammonium

NH_4^+ concentrations were determined using the methods of Karoleff (1976), in which the NH_4^+ ion was reacted with phenol and hypochlorite in the presence of nitro-prusside to produce irido-phenol blue, whose concentration was determined spectrophotometrically on a Pye-Unicam spectrophotometer with a path length of 1cm at 520nm.

2.2.3 Bacterial numbers

Total count was estimated using the A.O.D.C. method (Hobbie et al., 1977). Cells were differentially counted, using a Leitz Laborlux U.V. fluorescent microscope and assigned to the following morphotypes; described by Painting et al. (1985).

Small cocci	:	0.2 - 0.3µm
Large cocci	:	0.31 - 0.8µm
Small rods	:	0.8 - 1.2µm
Large rods	:	1.21 - 2.0µm
Small microcycloid	:	0.8 - 1.2µm
Large microcycloid	:	1.2 - 2.0µm
Spirillar	:	0.8 - 1.2µm

The total count was determined using the following formula:

$$N = \frac{S \times 10^6 \times n}{s \times v} \text{cells.m}\ell\text{-1}$$

where S = working surface area of the filter (mm^2)

n = mean number of cells.field^{-1}

s = area of microscopic field (μm^2)

v = volume of sample filtered (mℓ)

In each case, at least 300 cells were counted, and the volume filtered was adjusted so that there were 15 - 30 cells.field^{-1}, as recommended by Linley (1983).

2.2.4 Biomass

The total biomass was calculated by reference to the differential counts estimated by A.O.D.C., using the following mean biovolumes described by Painting et al. (1985).

Small cocci	:	0.009 μm^3
Large cocci	:	0.142 μm^3
Small rods	:	0.198 μm^3
Large rods	:	0.672 μm^3
Small microcycloid	:	0.198 μm^3
Large microcycloid	:	0.672 μm^3
Spirillar	:	0.198 μm^3

Bacterial biomass was calculated using a specific gravity of 1.1g.cm^{-3} (Doetsch and Cook, 1973), a dry weight/wet weight ratio of 0.2 (Luria, 1960) while the carbon content was estimated as 50% of the dry weight (Sorokin and Kadota, 1972).

2.2.5 Viable count and percentage plateability (%P)

0.1mℓ aliquots were plated in triplicate on 0.1% Peptone Seawater agar (Pep-SWA) and 0.5% Pep-SWA (see Appendix), either undiluted or at an appropriate dilution to produce approximately 200 colonies per plate. Plates were incubated at 22°C for 10 days or until no further increase in colony formation occurred. Plates were counted using a Nikon 6CTC Profile Projector, at 10x magnification, which permitted the inclusion of very small colonies (<0.5mm). The %P was calculated by reference to the total count, determined by A.O.D.C., using the following formula:

$$\%P = \frac{V.C.}{T.C.} \times 100$$

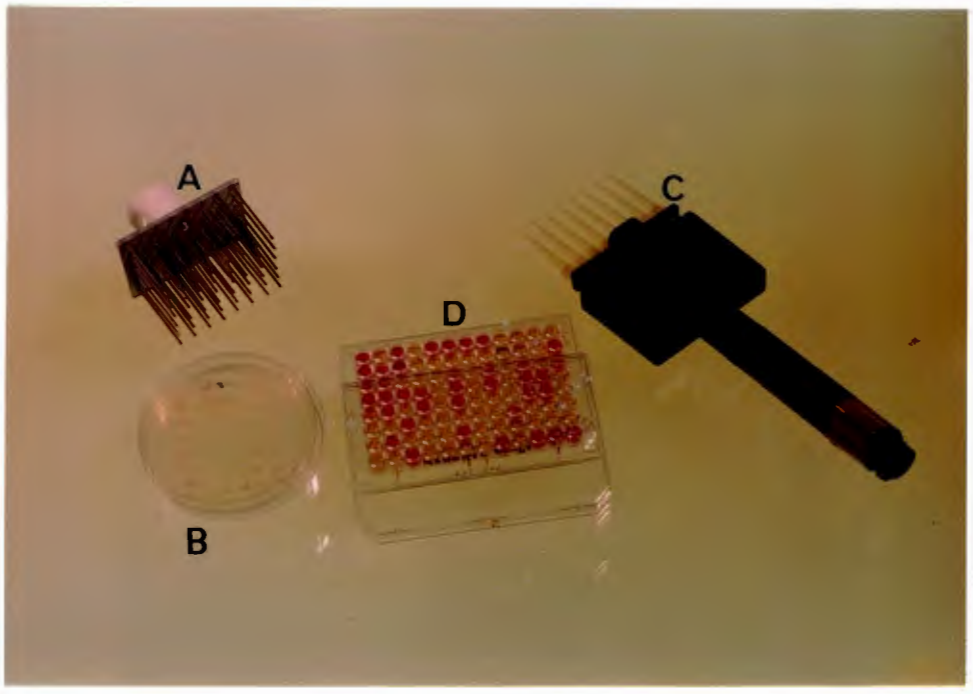

FIGURE 2: Apparatus used in characterisation of plateable bacterial populations. A: 48 pronged inoculator; B: 0.5% Peptone-Seawater Agar plate with 48 randomly picked isolates; C: Titertek 8-channel automatic pippetter; D: Nunclon Micro-titer tray.

where V.C. = Viable Count (CFU.mℓ$^{-1}$)

T.C = Total Count (Cells.mℓ$^{-1}$)

2.2.6 Plateable population characteristics

For each of 9 days during Summer (days 15, 17, 23, 26, 31, 33, 35, 37 and 38) and 10 days during winter (days 10, 16, 17, 20, 22, 23, 25, 27, 28, 29), colonies were randomly picked from 0.1% Pep-SWA plates to determine the population characteristics. In each case, a line was drawn across the plate and colonies on or within 1mm of the line were picked with sterile toothpicks and streaked onto 0.5% Pep-SWA. During the summer sampling, 40 colonies were selected, but the number was increased to 48 during the winter sampling. All colonies were re-streaked to purity, and the collection for each day was then spotted onto a single 0.5% Pep-SWA master plate in a 6 x 8 array (fig.2). This was then incubated at 22°C for 7 days, whereafter it was stored at 4°C. All subsequent transfers of colonies and inoculations were done using a 48 pronged inoculator (fig.2) consisting of 48 stainless steel prongs 4cm in length and 1mm in diameter set in an aluminium base plate, which forms a 4.5cm x 6.25cm array corresponding exactly to the format of the colony array on inoculated plates. The inoculator was used to effect inoculation of both solid and liquid media.

It was found that the loss of isolated strains occurred from the collections on master plates, particularly during the summer sampling period. Once such losses had occurred, it was not possible to replace them, since this would have introduced a selective bias into the collection, favouring easily maintained strains.

Isolated colonies were submitted to 20 basic identification tests. Test inoculations were done from an overnight broth culture in 0.5% Pep-SWA (see Appendix) inoculated from the master plate. All broth assays were done in standard Nunclon[C] micro-titer assay trays (fig.2). These have 96 wells, so that two 6 x 8 format inoculations could be run at the same time in each tray: wells were filled with 0.15mℓ of the appropriate broth, using a Titertek[C] 8 channel multi-pipette. The following characterisation tests were performed:

Tests on solid media

2.2.6.1 Colony colour

A 0.5% Pep-SWA plate was inoculated and incubated for 5 days at 22°C: Cytophaga/Flavobacterium isolates were characterised by their chromogenic nature.

2.2.6.2 Oxidase reaction

The same plate used for colony colour determination was used to determine the oxidase reaction. The plate was flooded with oxidase reagent (see Appendix) and then drained. Production of a deep blue colour within 30 sec was taken to be a positive reaction.

2.2.6.3 Growth at 30°C

A 0.5% Pep-SWA plate was inoculated and incubated at 30°C for 48 hours, and then checked for presence and extent of growth of each colony. Growth was rated as nil, moderate (+), or extensive (++).

2.2.6.4 Growth at 37°C

A 0.5% Pep-SWA plate was inoculated and incubated at 37°C for 48 hours, and then checked as above. Growth was rated as nil, moderate (+) or extensive (++).

2.2.6.5 Agarase production

A 0.5% Pep-SWA plate was inoculated and incubated at 22°C for 48 hours, and then checked for pitting by agarase producers. The plate was then flooded with Gran's reagent (see Appendix) and zones of clearance, which

indicate agarase production, were noted. Two types of agarase were recognised: the first produced a clearly defined ring of clearance, while the second produced a diffuse zone of clearance. Any positive agarase production was further characterised by these and the extent of agarolytic activity was defined as negative, moderate (zone <1.5 x colony diameter) or extensive (zone >1.5 x colony diameter).

2.2.6.6 Growth on Thiosulphate-Citrate-Bile salt agar (T.C.B.S.)

A T.C.B.S. plate (see Appendix) was inoculated and incubated at 22°C for 7 days. Colony growth and characteristic colony colour were noted. Growth was rated as positive or negative.

2.2.6.7 Growth on seawater Cetrimid plates

A seawater Cetrimid agar plate (see Appendix) was inoculated and incubated at 22°C for 7 days, and colony growth was rated as positive or negative.

2.2.6.8 Caseinase production

A seawater skim milk agar plate (see Appendix) was inoculated and incubated at 22°C for 48 hours.

Caseinase production resulted in clear zones around colonies, which were rated as moderate (<1.5 x colony diameter), or extensive (>1.5 x colony diameter).

2.2.6.9 Gelatin liquefaction

A gelatin seawater agar plate (see Appendix) was inoculated and incubated at 22°C for 24 hours. The plate was then flooded with Frazier's reagent (see Appendix). A positive reaction was indicated by clear zones around active colonies. Activity was rated as moderate (<1.5 x colony diameter) or extensive (>1.5 x colony diameter).

2.2.6.10 Cellulase production

Cellulase production was inferred by the assessment of carboxymethylcellulose (C.M.C.) hydrolysis on C.M.C. seawater agar plates (see Appendix). Plates were inoculated and incubated at 22°C for 24 hours. Thereafter, colonies were washed off the plate with a 1M NaCl solution, and the plate was flooded with a 1% Congo-Red solution in distilled water. Plates were stained for 15m after which they were washed with a 1M NaCl solution and fixed by flooding with a 0.25N solution of HCl. Cellulase production was inferred by clear zones around or below colonies. Activity was rated as moderate

(<2 x colony diameter) or extensive (>2 x colony diameter). A second plate was inoculated and tested after 1 week, as some colonies produced a very slow positive reaction.

Tests in broth mixtures

2.2.6.11 **Oxidative acid production from glucose**

Colonies were inoculated into 0.15mℓ aliquots of glucose peptone seawater broth (see Appendix), held in sterile microtiter trays. Trays were incubated at 22°C for 12 hours, and acid production was indicated by the production of a straw-yellow colour on addition of a drop of 0.1% Phenol Red (in distilled water).

2.2.6.12 **Oxidative acid production from mannitol**

Colonies were inoculated into 0.15mℓ aliquots of mannitol peptone seawater broths (see Appendix) and incubated for 12 hours, whereafter they were tested with 0.1% Phenol Red as above.

2.2.6.13 **Fermentative acid production from glucose**

Colonies were inoculated into 0.15mℓ aliquots of glucose peptone seawater broth as above, but the broths

were incubated at 22°C for 48 hours in an anaerobic jar, flushed twice with N_2 for 20 min, in which a resazurin indicator (Biolab) was included to show complete anaerobiosis. Acid production was tested with 0.1% Phenol Red as above.

2.2.6.14 Fermentative acid production from mannitol

Colonies were inoculated into 0.15mℓ aliquots of mannitol-peptone seawater broth, and these were maintained under anaerobic conditions as above, after which they were tested for acid production by the addition of 0.1% Phenol Red.

2.2.6.15 Nitrate Reductase activity

Duplicate seawater NO_3 broths (see Appendix) were inoculated and incubated at 22°C for 24 hours: one was maintained under aerobic conditions and the other under anaerobic conditions as above. Broths were then tested with Griesler's reagents (see Appendix) to demonstrate any reduction of NO_3 to NO_2. The extent of NO_3 Reductase activity was rated as slight (pink or red) or extensive (deep purple, brown).

2.2.6.16 Nitrite Reductase

All colonies showing a negative reaction to Griesler's reagent (see above) were tested with a drop of powdered Zn suspended in 10% Glycerol. A negative reaction indicated that reduction of NO_2 had taken place, in which case the colony was designated Nitrate Reductase ++, Nitrite Reductase +.

2.2.6.17 Motility

While the use of motility as a discriminatory characteristic has been criticised (Bianchi and Bianchi, 1982), using the method described here it has been found to be a useful and reproducible characteristic. Motilities were tested using the initial overnight broth culture. Using a Titertek[C] 8-channel multi-pipette, up to 3 rows of hanging drops were mounted on a large (3.5cm x 6.5cm) coverslip supported by plasticine on a large microscope slide. This was viewed under phase-contrast at 1200x magnification.

2.2.6.18 Form and size

Cell form was determined from the hanging drops used to determine motility. Cell sizes were defined as large

(>1.2μm), medium (0.8-1.2μm), small (0.5-0.8μm) or very small (<0.5μm).

2.2.6.19 Gram stain

Gram stains were performed on inocula from the initial overnight broth culture. The 48 pronged inoculator was used to place drops on a large (5cm x 7cm) glass slide: these were then air dried and flame fixed prior to staining.

2.2.6.20 Catalase activity

Catalase activity was determined in 12 hour seawater peptone broth cultures. The length of incubation was carefully timed, as considerable variation was observed to occur depending on the length of incubation. A drop of 10% (v/v.) H_2O_2 was added to each well: a positive reaction was indicated by the production of bubbles of O_2 within 10 min., and the extent of activity was rated as slight, moderate or extensive.

2.2.7 Generic determination

A very simple classification schedule was used to assign tested strains to one of 5 generic groups, based on the schedule proposed by Oliver (1982). The terms oxidative

and fermentative are used in the sense defined by Cowan and Steel (1970). Oxidative strains are considered to be obligate aerobes which may produce acid from carbohydrates under aerobic, but never under anaerobic conditions. Fermentative strains were those which produced acid from carbohydrate under anaerobic conditions. In this instance these were all facultative anaerobes, which usually produced acid from carbohydrates under aerobic conditions as well.

Vibrio: Motile, facultative anaerobic (fermentative) isolates, generally oxidase positive, frequently (but not always) TCBS positive.

Enterobacteriaceae: Non-motile, facultative anaerobic isolates, generally oxidase negative.

Pseudomonas: Motile, obligately aerobic (oxidative) isolates, but which did not necessarily produce acid from carbohydrates under aerobic conditions. Generally oxidase positive.

Acinetobacter: Non-motile, oxidative isolates, generally oxidase negative.

Cytophaga/Flavobacterium: Chromogenic (yellow, orange or red) isolates, which were non-motile or (rarely) showed gliding motility. Oxidative, and only rarely produced acid from carbohydrates. Oxidase negative.

2.2.8 Statistical analysis

2.2.8.1 Multivariate analysis

All isolates were submitted to 20 discriminatory tests. As certain of the tests were further defined by the extent of a positive reaction, colour reactions, etc., a maximum total of 32 bits of information could be assigned to any one isolate (see Table 4). In this instance, the Gram reaction was eliminated from subsequent analysis, since no Gram positive strains were isolated. As a result all multivariate analyses were based as a data matrix comprising 31 possible characters. A moderate weighting system was applied, as advised by Field et al. (1982). In those tests in which a positive grading was applied (e.g. agarase, catalase production), the tests had an inherent maximum score of 2 (-ve reaction = 0, moderate +ve reaction = 1, extensive +ve reaction = 2), while in other tests in which only a positive or negative result was possible (colony colour, oxidase reaction, motility, etc.) a positive result was given a weighted score of 2 in order to avoid a bias towards the

former reactions. Other tests (form and size, agar pit presence, agarase type) were relatively less important in the discrimination analyses and were left unweighted with a maximum score of 1.

These data were analysed using a multivariate analysis technique described by Field *et al.* (1982) which constructs a similarity matrix using the Bray-Curtis measure of similarity, which is defined as:

$$\delta_{jk} = \frac{\sum_{i=1}^{s} \left| Y_{ij} - Y_{ik} \right|}{\sum_{i=1}^{s} \left| Y_{ij} + Y_{ik} \right|}$$

where Y_{ij} = score for the i^{th} species in the j^{th} sample

Y_{ik} = score for the i^{th} species in the k^{th} sample

δjk = dissimilarity between j^{th} and k^{th} samples, summed over s species.

δjk ranges from 0 (all scores identical) to 1 (all scores dissimilar), and is the complement of the similarity Sjk:

$$Sjk = 1 - \delta jk$$

Similarity scores are then sorted by a hierarchical strategy to produce a classification dendrogram (Clifford

and Stephenson, 1975). In this instance, this was done by the Group-Average Sorting method, which joins 2 groups of samples together at the average level of similarity between all the members of the one group and all the members of the other.

The use of this method has certain disadvantages, however: the hierarchical arrangement of groups is irreversable and once assigned to a group, the sample or species is lost; the dendrogram displays only inter-group relationships and the analysis is thus based on average intergroup values, and the dendrogram tends to over emphasize discontinuities and may thus force a graded series into separate groups (Field et al., 1982). As a result, a complementary method was used to confirm relationships suggested by the classification dendrogram. In this instance, multi-dimensional scaling (MDS) was used to provide a 2-dimensional ordination of each similarity matrix, as described by Kruskal and Wish (1978) and Field et al. (1982), in which the distances between points are proportional to the degree of dissimilarity between strains tested.

Multivariate analyses (classification dendrogram and MDS) were performed on 2 sets of data: for each of 5 selected days during the summer sampling period (days 18, 20, 33, 35 and 37 of sampling) and 5 during the

winter sampling period (days 10, 22, 23, 27 and 28 of sampling) the data for the entire selected bacterial population were analysed to define groups used in diversity analyses, and a second set of analyses (classification dendrograms only) were performed on summary data matrices for all days tested during summer and winter (Tables 2, 3).

2.2.8.2 Diversity

For each of the 5 selected days during summer and winter for which classification dendrograms were computed, the Shannon-Weaver Diversity Index (H') was determined, following the methods of Bianchi and Bianchi (1982), using the expression

$$H' = -\sum_{i=1}^{N} Pi \log_2 Pi$$

where Pi is the proportion of the total number of strains in the i^{th} group.

Groups were defined at the 85%S level.

In addition a complementary, simple expression of diversity (D) was determined in which the number of groups present at the 85%S level (N) was determined as a percentage of the total number of groups possible (which = n)

$$D = {}^{N}/_{n} \times 100$$

2.3 Results

2.3.1 Wind

2.3.1.1 Summer

During summer, the wind was most commonly an off-shore south-easterly (fig.3a). It was observed that strong south-easterly winds which blew for more than 2 days were strongly correlated with the occurrence of upwelling, as described by Andrews and Hutchings (1980) and Jury et al. (1985a, b).

Such upwelling episodes were either followed by periods of low wind velocity, or more frequently by the onset of onshore winds, which resulted in downwelling of surface waters at the coast.

2.3.1.2 Winter

During winter, winds were most commonly onshore north-westerlies (fig.3b). During the sampling period winds were commonly of low velocity ($<10 km.h^{-1}$) but 6 periods of storm conditions occurred on days 3-4, 6, 11, 16-18, 20 and 23.

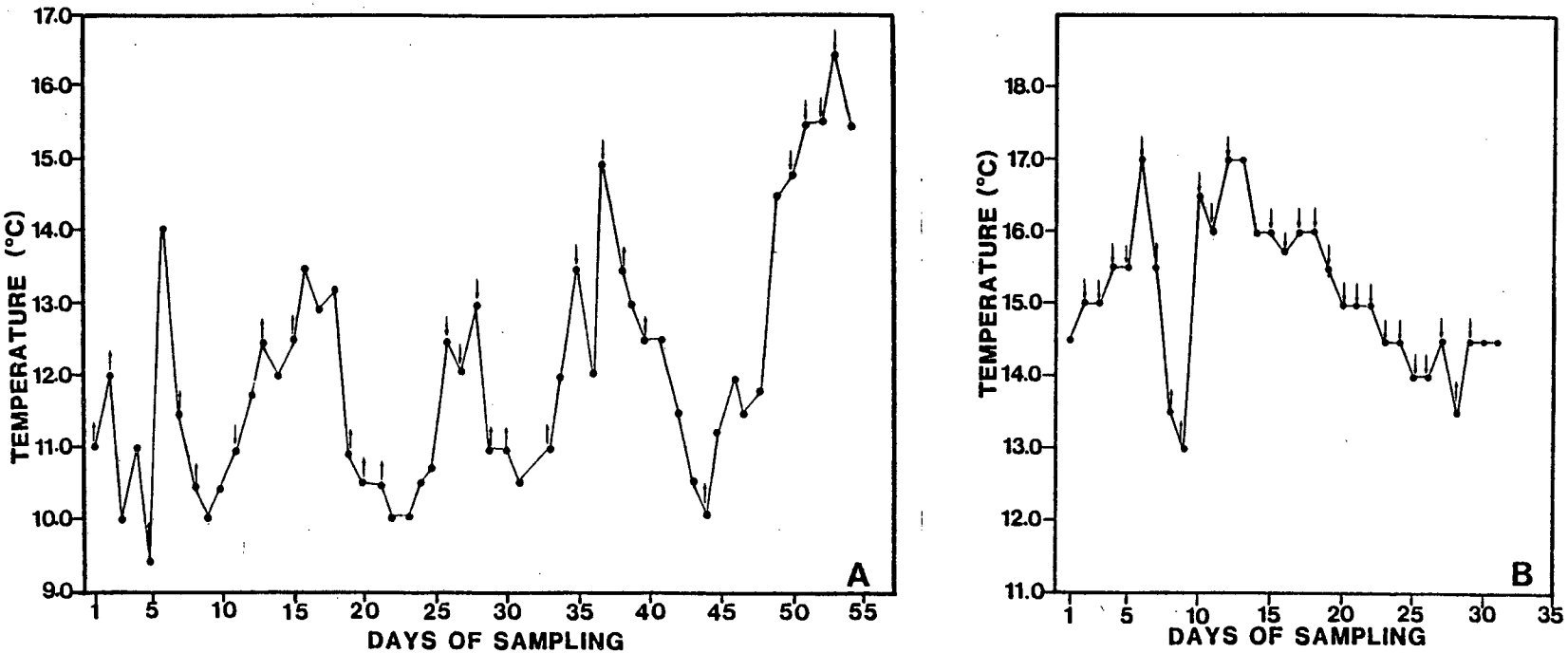

FIGURE 3: Daily water temperatures recorded at Oudekraal (Fig. 3a: Summer sampling period; Fig. 3b: Winter sampling period. ↑ - offshore wind > 10 $km.h^{-1}$. ↓ - onshore wind > 10 $km.h^{-1}$).

Two periods of strong south-easterly winds occurred on days 7-9 and 28. The first induced a strong upwelling between days 8 and 10, and the second produced a weak and brief upwelling on day 29.

2.3.2 Water temperature

2.3.2.1 Summer

Water temperatures for the summer sampling period are shown in fig.3a. Temperatures varied widely throughout the sampling period, ranging from 9.5°C to 16.5°C (mean = 12.1°C, s = 1.66°C). There was a distinct periodicity in this variation, and periods of low temperature (<11.0°C), thought to be characteristic of upwelling, alternated with periods of high water temperature at approximately 5 day intervals (mean = 4.94d); 5 such alternative upwelling and downwelling cycles were noted.

2.3.2.2 Winter

Water temperatures for the winter sampling period are shown in fig.3b. The mean winter temperature (15.50°C, s = 1.04°C) was higher than that recorded during summer, and temperatures ranged between 13°C and 17°C.

TABLE 1A

Correlation of hydrological, nutrient and bacteriological parameters during the 54 day summer sampling period at Oudekraal. Values are for the expression y = ax + b . r = correlation efficient, n = sample size and p = probability. Statistically significant correlations are marked *.

x	y	a	b	r	n	p
Temp.	NO_3 conc	36.29	-2.22	-0.57	52	<0.01*
"	NO_2 conc	0.66	-0.02	-0.16	S2	>0.1
"	NH_4 conc	2.38	-0.09	-0.25	52	>0.1
"	Urea conc	4.34	-0.18	-0.08	42	>0.1
"	$\frac{NO_3+NO_2}{NH_4^+ + Urea}$	15.21	-1.01	-0.50	49	<0.01*
"	$\frac{NO_3+NO_2}{Total\ N}$	1.46	-0.07	-0.59	49	<0.01*
"	Bacterial numbers	1.17×10^6	1.41×10^6	0.56	54	<0.01*
"	Bacterial biomass	-0.44	0.56	0.55	54	<0.01*
"	%P (0.1% Pep-SWA)	0.56	-0.03	-0.34	54	<0.1
$\frac{NO_3+NO_2}{NH_4+Urea}$	Bacterial numbers	5.32	-0.50	0.46	49	<0.01*
$\frac{NO_3+NO_2}{N}$	Bacterial numbers	12.68	-13.52	-0.68	49	<0.01*
$\frac{NO_3+NO_2}{NH_4+Urea}$	Bacterial biomass	0.32	-0.02	0.52	51	<0.01*
$\frac{NO_3+NO_2}{N}$	Bacterial biomass	0.68	-0.69	-0.76	51	<0.01*
$\frac{NO_3+NO_2}{NH_4+Urea}$	%P (0.1% Pep-SWA	0.15	-0.01	0.28	48	>0.1
$\frac{NO_3+NO_2}{N}$	%P (0.1% Pep-SWA	0.07	-0.41	0.50	48	<0.01*

TABLE 1B

Correlation of hydrological, nutrient and bacteriological parameters during the 31 day winter sampling period at Oudekraal. Values are for the expression $y = ax + b$. r = correlation efficient, n = sample size and p = probability. Statistically significant correlations are marked *.

x	y	a	b	r	n	p
Temp.	NO_3 conc	35.94	-1.91	-0.73	27	<0.001*
"	NO_2 conc	0.04	-0.04	-0.10	27	>0.1
"	NH_4 conc	2.86	-0.011	-0.01	27	>0.1
"	Urea conc	1.40	- .017	-0.02	27	>0.1
"	$\frac{NO_3+NO_2}{NH_4^+ + Urea}$	8.82	-0.47	-0.62	27	<.001*
"	$\frac{NO_3+NO_2}{Total\ N}$	1.52	-0.06	-0.65	27	<.001*
"	Bacterial numbers	10.74	-0.57	.39	30	<0.05>.02*
"	Bacterial biomass	.23	- .008	- .16	30	>0.1
"	%P (0.1% Pep-SWA)	0.12	-0.006	-0.03	28	>0.1
$\frac{NO_3+NO_2}{NH_4+Urea}$	Bacterial numbers	1.78	-0.071	0.071	27	>0.1
$\frac{NO_3+NO_2}{N}$	Bacterial numbers	.04	3.146	0.22	27	>0.1
$\frac{NO_3+NO_2}{NH_4+Urea}$	Bacterial biomass	.11	-0.007	-0.01	27	>0.1
$\frac{NO_3+NO_2}{N}$	Bacterial biomass	.079	.033	0.06	27	>0.1
$\frac{NO_3+NO_2}{NH_4+Urea}$	%P (0.1% Pep-SWA	.216	.050	0.15	27	>0.1
$\frac{NO_3+NO_2}{N}$	%P (0.1% Pep-SWA	.28	.03	0.276	27	>0.1
$\frac{NO_3+NO_2}{NH_4+Urea}$	%P (0.5% Pep-SWA	.20	.017	.060	27	>0.1
$\frac{NO_3+NO_2}{N}$	%P (0.5% Pep-SWA	.22	0.02	.008	27	>0.1

Three periods of low water temperature were recorded: the first occurred as a result of upwelling induced by strong south-easterly winds between days 8 and 10, the second was the result of cooling of waters during an extended period of cloudy weather between days 18 and 25, and the third was the result of a brief, weak upwelling which occurred between days 27 and 29.

2.3.3 Nutrients

2.3.3.1 Nitrate

2.3.3.1.1 Summer

The results of determinations of NO_3 concentrations are presented in fig.4a. NO_3 concentrations varied widely throughout the sampling period, with a maximum recorded value of 29.98µg at NO_3-N.l^{-1} and a minimum of 0.93µg at NO_3-N.l^{-1} (mean = 9.32µg at NO_3-N.l^{-1} s = 6.64µg at NO_3-N.l^{-1}).

There was a marked periodicity in the occurrence of high NO_3 concentration: 5 peaks in NO_3 concentration occurred, which alternated with periods of low NO_3 concentration over a cycle of approximately 5 days.

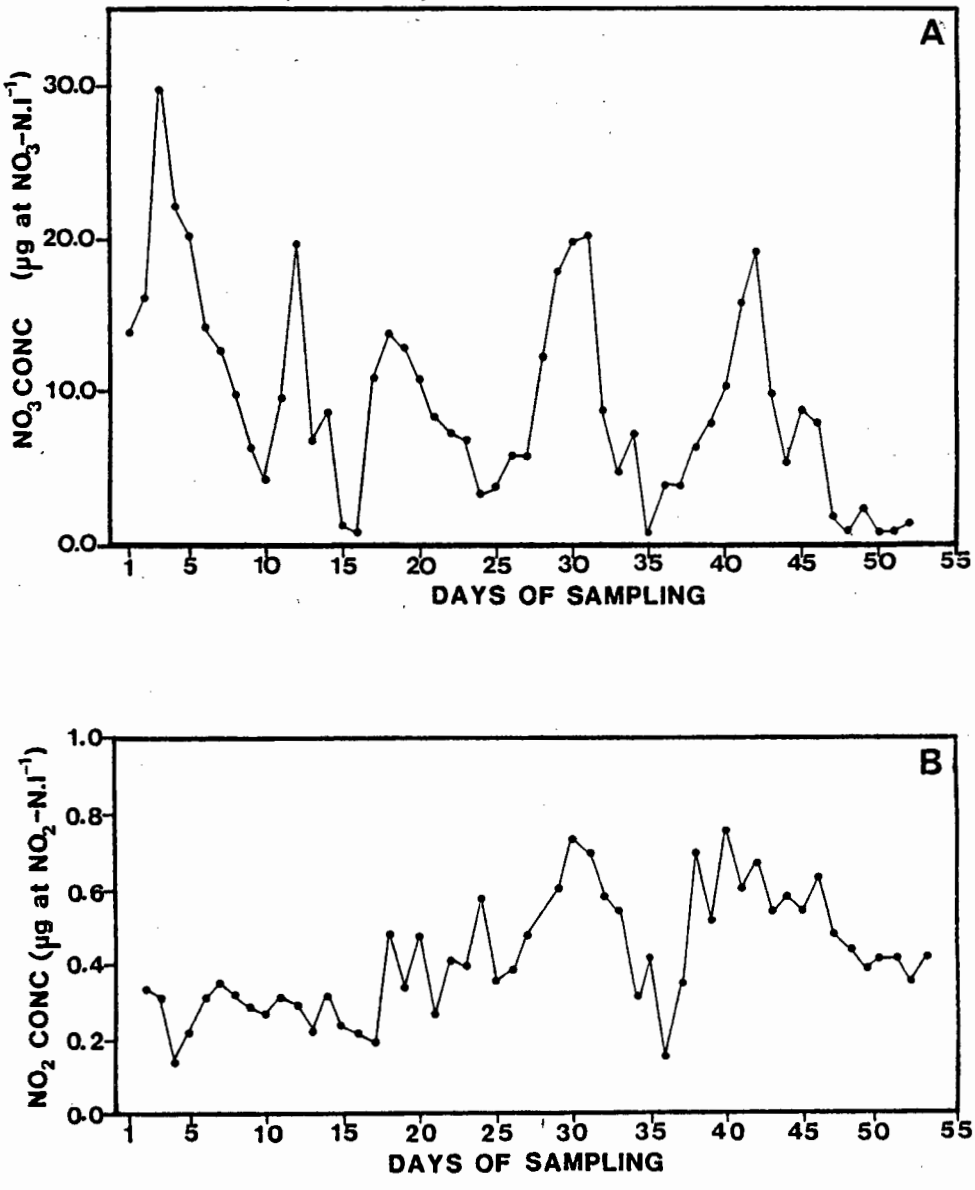

FIGURE 4: Concentration of nitrogen as nitrogenous compounds at Oudekraal during the summer sampling period. (Fig. 4a: NO_3-N concentration (µg at NO_3-N.l^{-1}). Fig. 4b: NO_2-N concentration (µg at NO_2-N.l^{-1}).

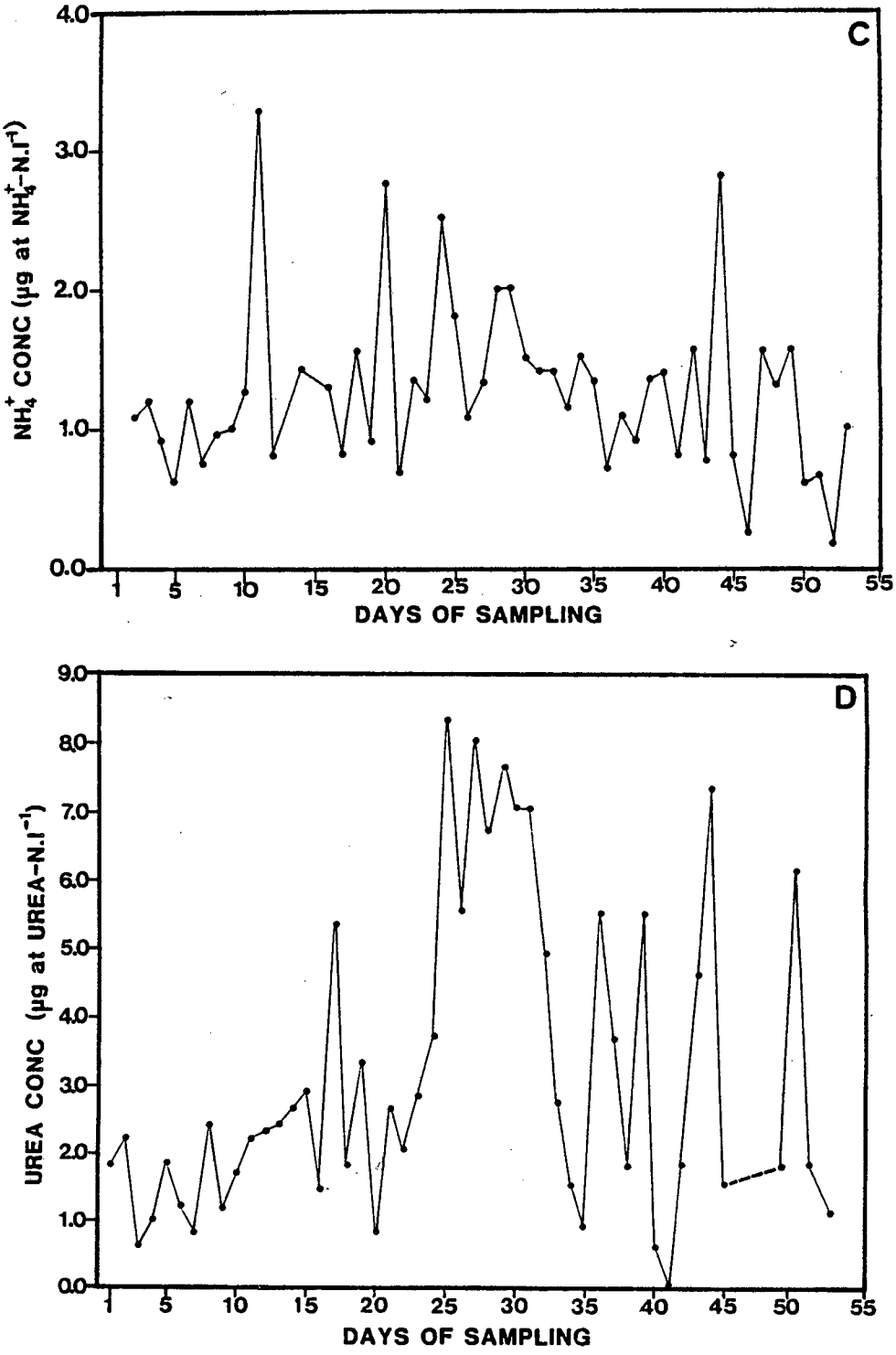

FIGURE 4: Concentration of nitrogen as nitrogenous compounds at Oudekraal during the summer sampling period. Fig. 4c: NH_4^+-N concentration (µg at NH_4^+-N.l^{-1}). Fig. 4d: Urea-N concentration (µg at Urea-N.l^{-1}).

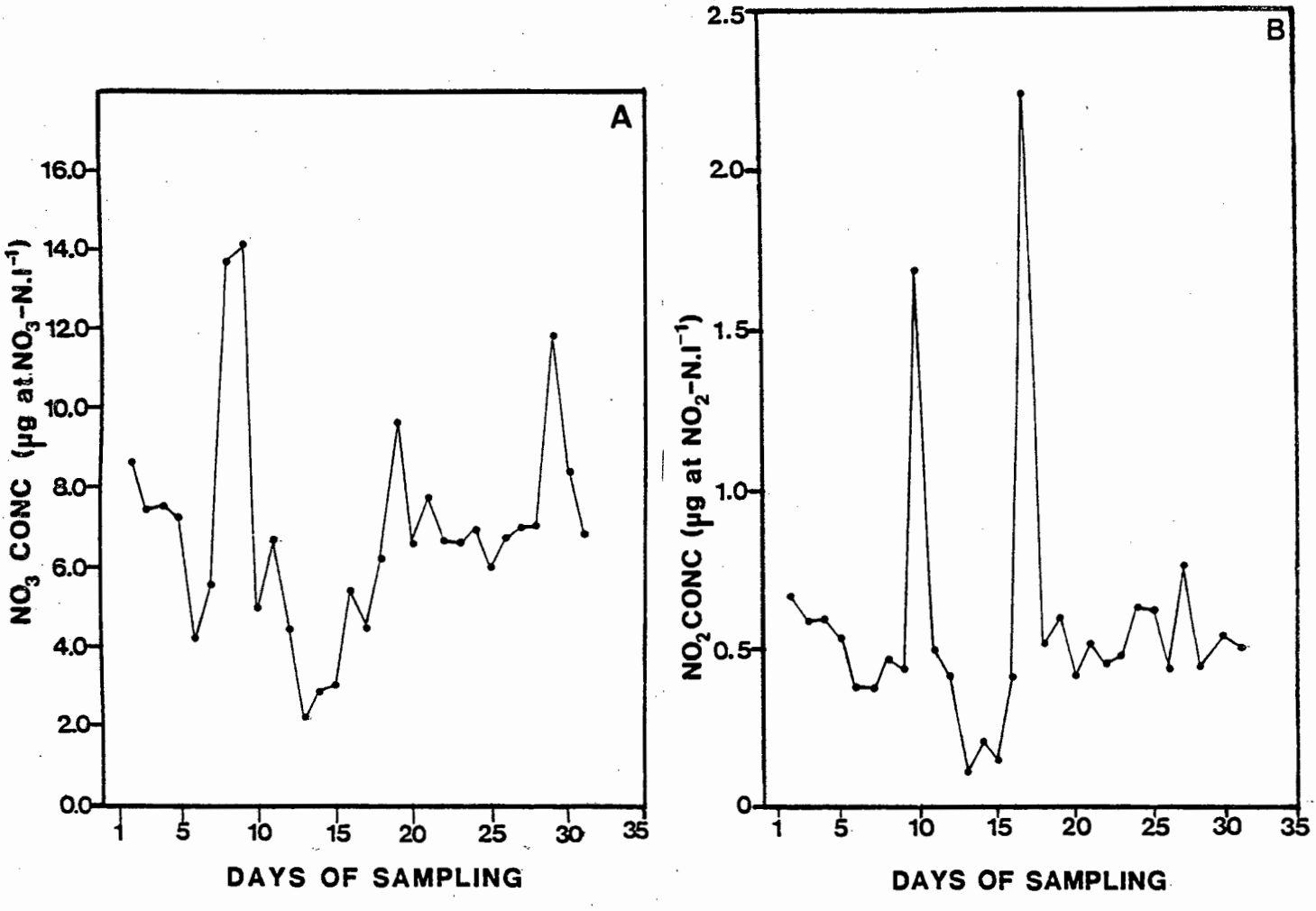

FIGURE 5: Concentration of nitrogen as nitrogenous compounds at Oudekraal during the winter sampling period. (Fig. 5a: NO_3-N concentration (µg at NO_3-N.l^{-1}). Fig. 5b: NO_2-N concentration (µg at NO_2-N.l^{-1}).

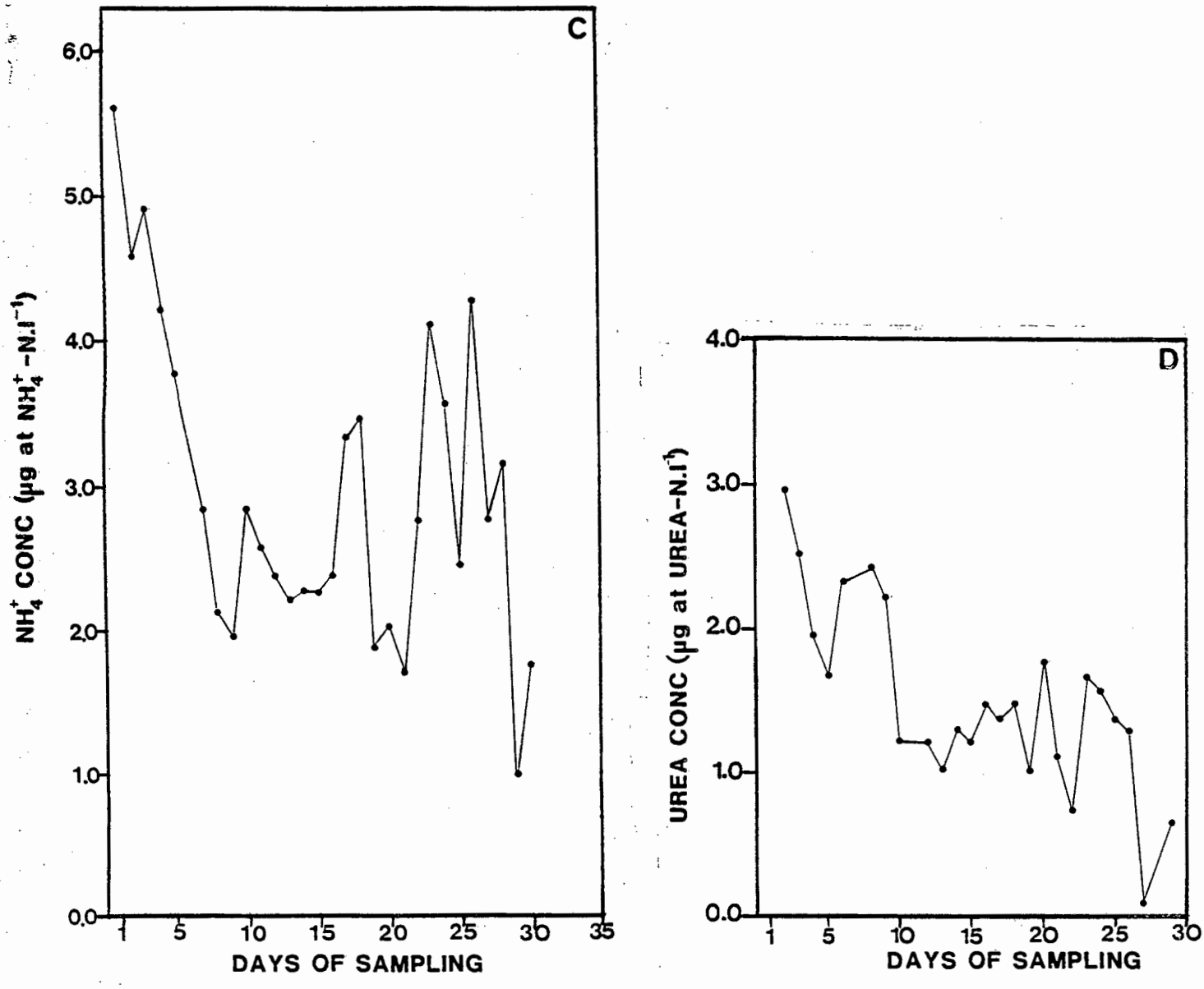

FIGURE 5: Concentration of nitrogen as nitrogenous compounds at Oudekraal during the winter sampling period. (Fig. 5c: NH_4^+-N concentration (µg at NH_4^+-N.l^{-1}). Fig. 5d: Urea-N concentration (µg at Urea-N.l^{-1}).]

With the exception of an anomolous warm water episode between days 4 and 8 (believed to have arisen from sun-warming of newly upwelled water) there was a strong negative correlation between water temperature and NO_3 concentrations (Table 1a). This agrees with the findings of Andrews and Hutchings (1980) which show that upwelling of South Atlantic Central Water is usually characterised by high concentrations of NO_3.

2.3.3.1.1 Winter

NO_3 concentrations for the winter sampling period are shown in fig.5a. The mean recorded NO_3 concentration (6.94µg at NO_3-N.l^{-1} s = 2.74µg at NO_3.N.l^{-1}) was lower than that recorded during summer.

Three episodes of elevated NO_3 concentrations occurred: the first was associated with upwelling which occurred between days 8 and 10, but the second correlated with the onset of winter storms between days 16 and 20. The third NO_3 elevation was associated with the brief, weak up welling which occurred between days 27 and 29.

A strong negative correlation between water temperature and NO_3 concentrations was present (Table 1b).

2.3.3.2 Nitrites

2.3.3.2.1 Summer

NO_3 concentrations for the summer sampling period are presented in fig.4b. There was some evidence of periodicity, but only a weak negative correlation was noted between water temperaure and NO_2 concentrations, which was not significant at the 1% level (Table 1a).

The mean NO_2 concentration recorded was 0.41µg at NO_2-N.l^{-1} (s = 0.15µg at NO_2-N.l^{-1}).

2.3.3.2.2. Winter

NO_2 concentrations for the winter sampling period are presented in fig.5b. The concentration of NO_2 was generally lower that that recorded during summer (mean = 0.58µg at NO_2-N.l^{-1}, s=0.42µg at NO_2-N.l^{-1}). Two concentrations >1.5µg NO_2-N.l^{-1} were noted on days 11 and 18, which corresponded with the occurrence of North- West wind storms.

No significant correlation was found between water temperature and the concentration of NO_2 (Table 1b).

2.3.3.3 Ammonia

As discussed by Goering et al. (1964) and Eppley et al. (1971), the determination of NH_4^+ concentrations proved difficult as wide variations in recorded levels were observed due to rapid fluxes through the planktonic population.

2.3.3.3.1 Summer

NH_4^+ concentrations for the summer study period are presented in fig.4c. There was no evidence of any periodicity in the occurrence of NH_4^+, and the mean recorded value was 1.35µg at NH_4^+-N.l^{-1} (s = 0.75µg at NH_4^+-N.l^{-1}).

No correlation could be drawn between the water temperatures and NH_4^+ concentrations (Table 1a).

2.3.3.3.2 Winter

NH_4^+ concentrations for the winter study period are pre- sented in fig.5c. The initial concentrations were high (5.62µg at NH_4^+-N.l^{-1}) falling over a period of 8 days to 2.0µg at NH_4^+-N.l^{-1}, whereafter the concentration was variable, but uncorrelated to changes in temperature changes (Table 1b).

The mean recorded NH_4^+ concentration in winter (2.94µg at NH_4^+-N.l^{-1}, s=0.08µg at NH_4^+.l^{-1}) was similar to that seen in summer.

2.3.3.4 Urea

2.3.3.4.1 Summer

Urea concentrations for the summer study period are presented in fig.4d. Wide variations in the concentration of urea were recorded, ranging from 0.09µg at urea-N.l^{-1} to 8.31µg at urea-N.l^{-1}. There was no evidence of periodicity in the occurrence of urea, and no correlation existed between urea concentrations and water temperature (Table 1a). The mean recorded concentration was 3.05µg at Urea-N.l^{-1} (s = 2.41µg at Urea-N.l^{-1}).

2.3.3.4.2 Winter

Urea concentrations for the winter study period are presented in fig.5d. The mean recorded concentration (1.70µg at Urea-N.l^{-1}) was lower than that recorded in summer, but there was less day-to-day variability in the urea concentrations (s = 0.98µg at Urea-N.l^{-1}). No correlation could be drawn between the concentration of urea and water temperature (Table 1b).

2.3.3.5 Total nitrogen and nitrogen ratios

Total recorded nitrogen levels (the sum of NO_3, NO_2, NH_4^+ & urea concentrations) for both summer and winter are presented in figs 6a and b respectively. Both NO_3 and NO_2 can be regarded as sources of "new" nitrogen, while NH_4^+ and urea represent "old" or recycled nitrogen (Dugdale and Goering, 1967; Herbert et al., 1977).

Despite the difficulties attached to measuring NH_4^+ and 60 urea levels (Goering et al., 1964; Eppley et al., 1971), the ratio between old and new nitrogen may provide an index of its biological history (Dugdale and Goering, 1967; Herbert et al., 1977; Probyn, 1985) and it can be hypothesised that the ratio of new:old nitrogen should rise in upwelled waters and fall in surface or downwelled waters which have a recent history of enhanced biological activity.

Two ratios were computed: the first was that of new nitrogen (NO_3 + NO_3) as a percentage of the total measured nitrogen, and the second was that of new:old nitrogen (NO_3 + NO_2:NH_4^+ + urea).

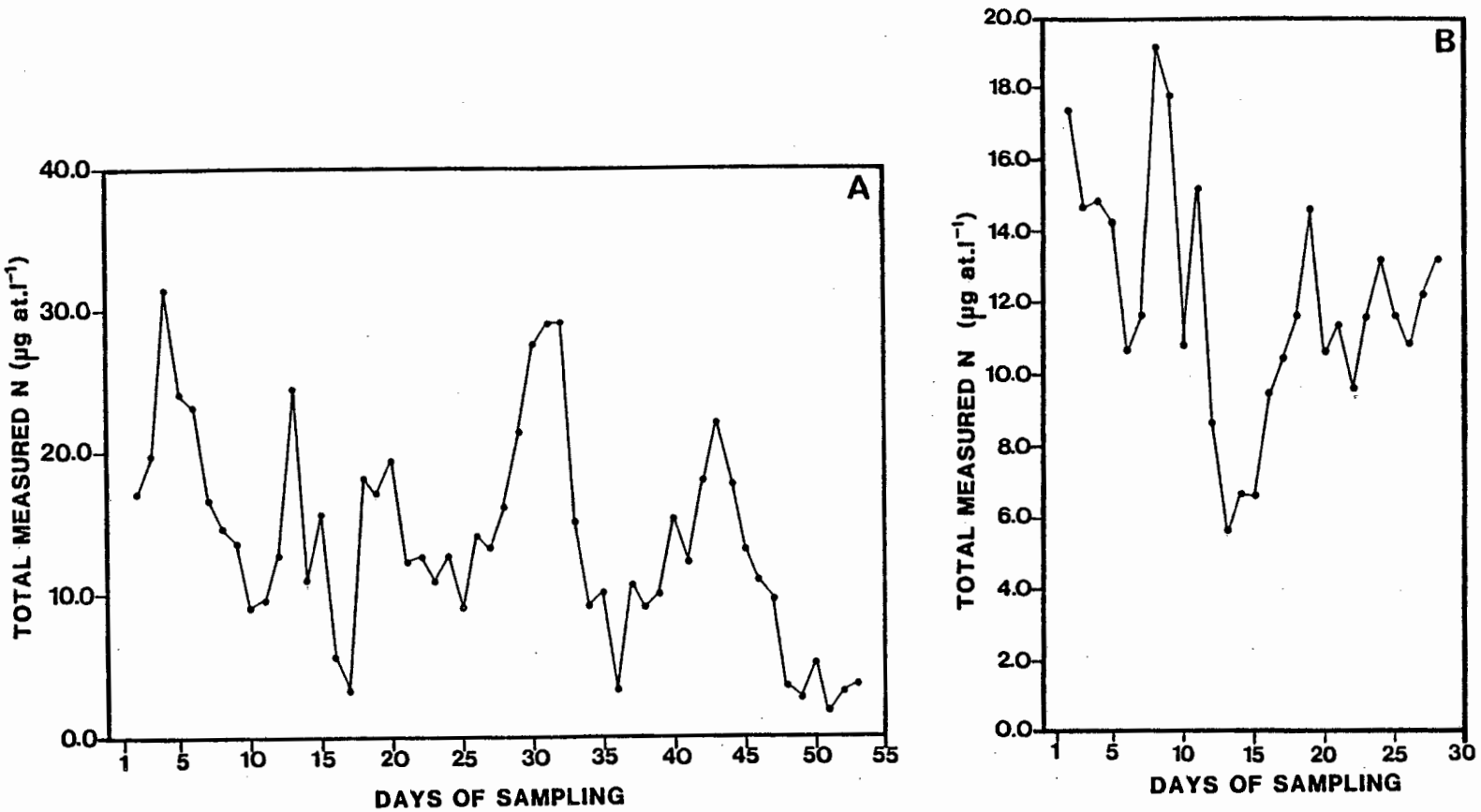

FIGURE 6: Total nitrogen concentrations in the water column at Oudekraal (µg at.l^{-1}).

(Fig. 6a: Summer sampling period. Fig. 6b: Winter sampling period).

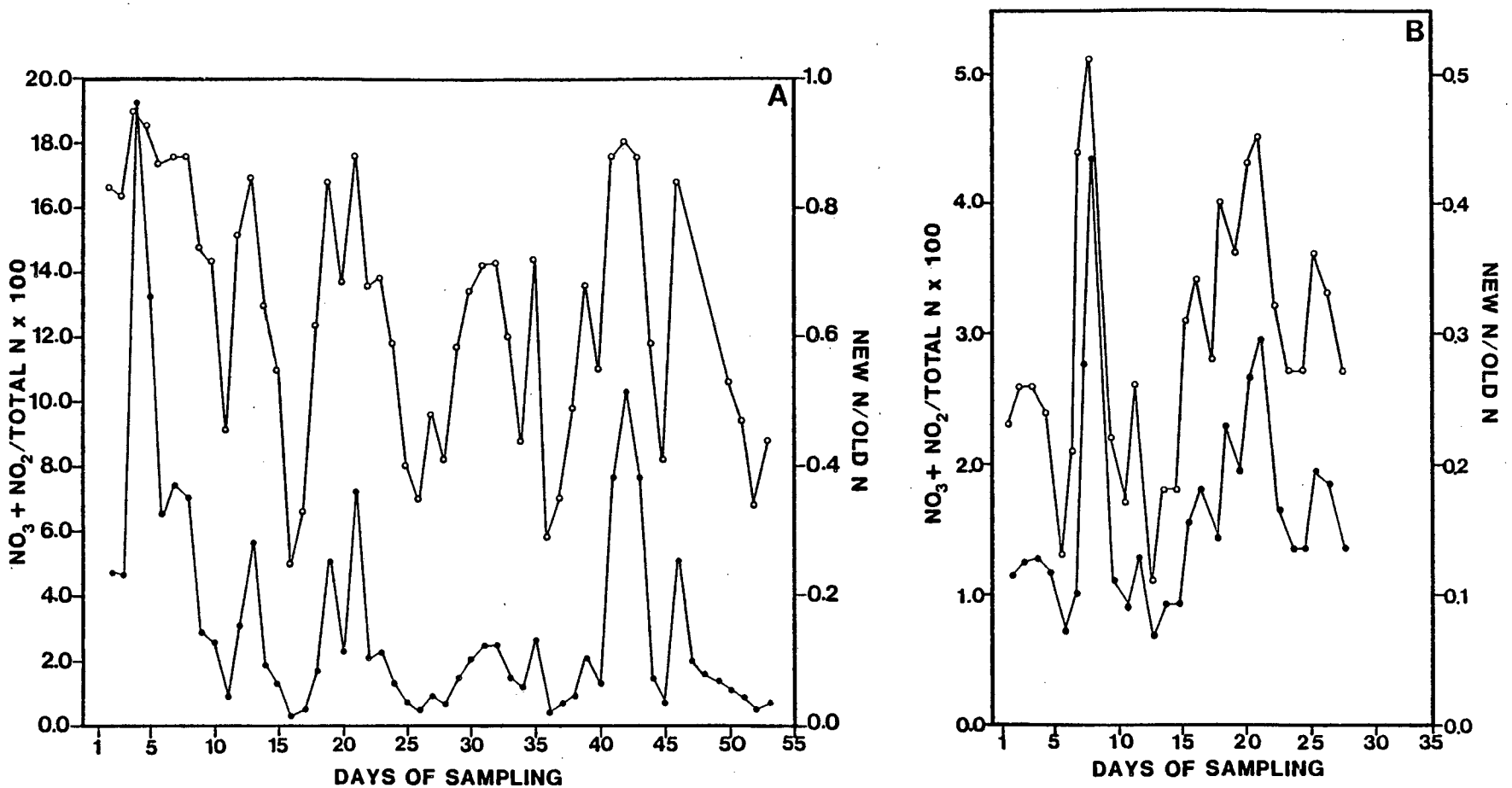

FIGURE 7: Ratios of New:Old nitrogen (——o——) and NO_3 + NO_2 (——●——) as a percentage of the total recorded nitrogen concentration. (Fig. 7a: Summer sampling period. Fig. 7b: Winter sampling period.)

2.3.3.5.1 Summer

Results of computation of both ratios for the summer study period are presented in fig.7a. There was a marked periodicity in both ratios, and a strong negative correlation between their values and water temperature was shown to be present (Table 1a), which confirmed the hypothesis that upwelling water represents the major source of new nitrogen in the system, and that old or recycled nitrogen is characteristic of aged upwelled waters.

One exceptional period of high water temperatures and high ratio values occurred between days 3 and 5 however, suggesting that water temperature is not always a reliable indicator of upwelling, and confirming the previous suggestion that upwelling water may sometimes be rapidly sunwarmed.

2.3.3.5.2 Winter

Results for both ratios for the winter period are presented in fig.7b. There was again a marked periodicity in the ratios which were strongly correlated with water temperature (Table 1b). Although both ratios were shown to increase in upwelling water (days 8, 9 and 29),

increases were also noted which coincided with the onset of onshore North-West wind storms.

2.3.4 Bacterial numbers

2.3.4.1 Summer

Total counts, computed from A.O.D.C., are presented in fig.8a. There was a marked periodicity in the numbers of bacteria recorded; numbers of bacteria were low in upwelling water and high in downwelling waters. In confirmation of this, strong positive correlations were noted between numbers of cells.mℓ$^{-1}$ and water temperature (Table 1a) and strong negative correlations existed between the total count and the ratios NO_3/Total N and NO_3 + NO_2/$NH4^+$ + urea. Very rapid changes in the total count thus occurred with changes in wind and hydrological conditions.

The mean total count recorded was 3.91 x 10^6 cells.mℓ$^{-1}$ (s = 3.70 x 10^6 cells.mℓ$^{-1}$), while the mean for days of upwelling (T < 11°C) was 1.64 x 10^6 cells.mℓ$^{-1}$ (s = 0.95 x 10^6 cells.ml^{-1}) and that of days of downwelling or where water temperature was >12°C was 5.98 x 10^6 cells.ml^{-1} (s = 4.19 x 10^6 cells.mℓ$^{-1}$). From these data it was evident that upwelling water presents a relatively constant, low

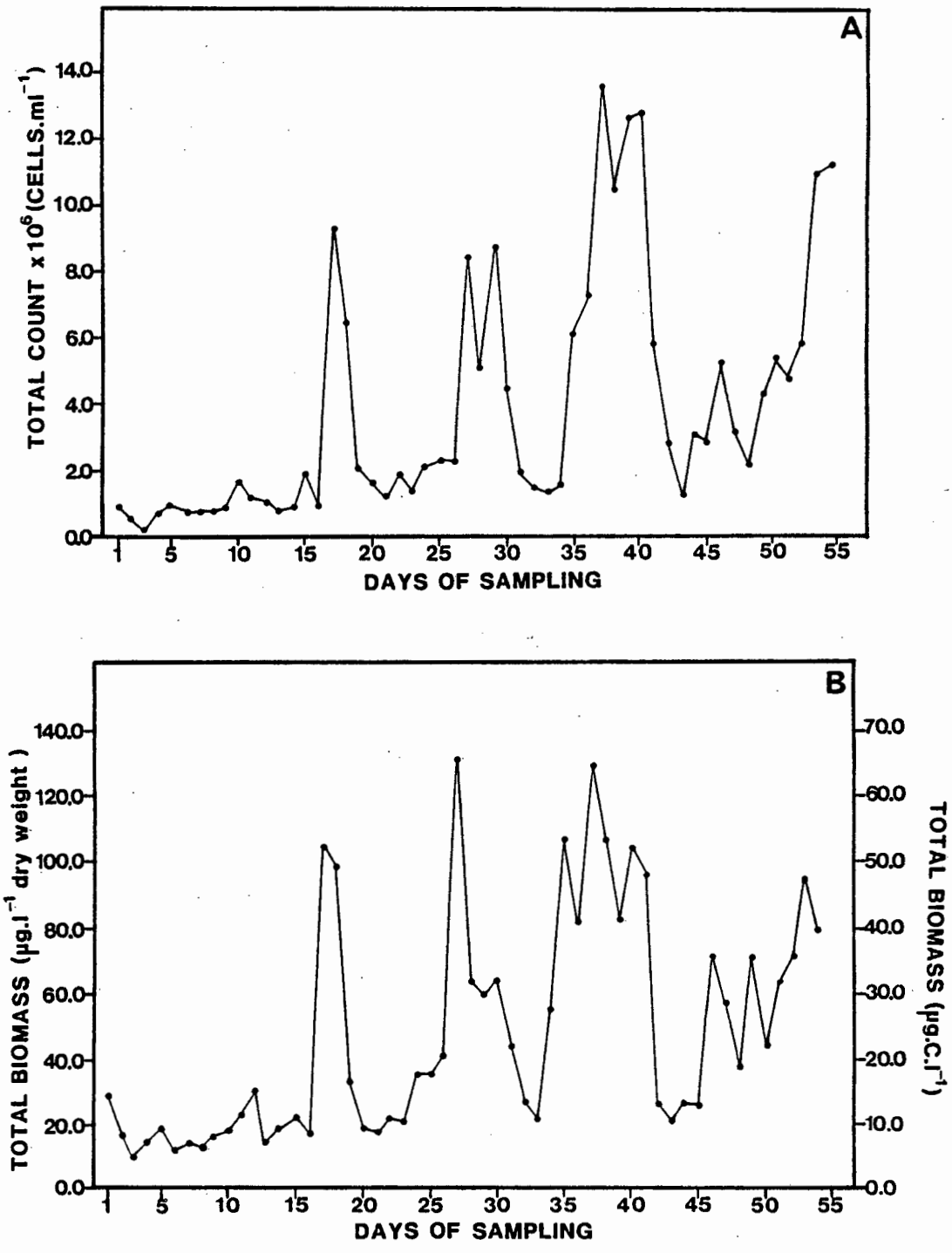

FIGURE 8: Total bacterial numbers (cells x 10^6.ml^{-1}) and biomass (µg. l^{-1} dry weight and µg bacterial C.l^{-1}) at Oudekraal during the summer sampling period. (Fig. 8a: Total count. Fig. 8b: Total biomass).

cell count. Subsequent to upwelling, however, the size of the populations of bacteria varied widely, but remained correlated to changes in water temperature and nutrient concentrations (Table 1a).

2.3.4.2 Winter

Results of total counts for the winter sampling period are presented in fig.9a. As in the summer sampling, large and rapid variations in observed total counts were noted, but for the whole period, the mean bacterial count was lower than that recorded in summer (mean = 2.16 x 10^6 cells.mℓ^{-1} s = 1.52 x 10^6 cells.mℓ^{-1}). The strong correlation recorded in summer between temperature and bacterial numbers was not observed during winter: although numbers did drop during the occurrence of upwelling, there was no rapid increase in numbers once upwelling ceased. In addition, there was no observed relationship between bacterial numbers and the nitrogen ratios (Table 1b). These effects are believed to be attributable to the fact that drops in water temperature were observed to occur with persistent onshore winds and winter storms; furthermore, the nitrogen content of the water remained fairly high throughout, and increases in the nitrogen concentration and the ratios NO_3/Total N and $NO_3 + NO_2/NH_4^+$ + urea were observed to occur which were not

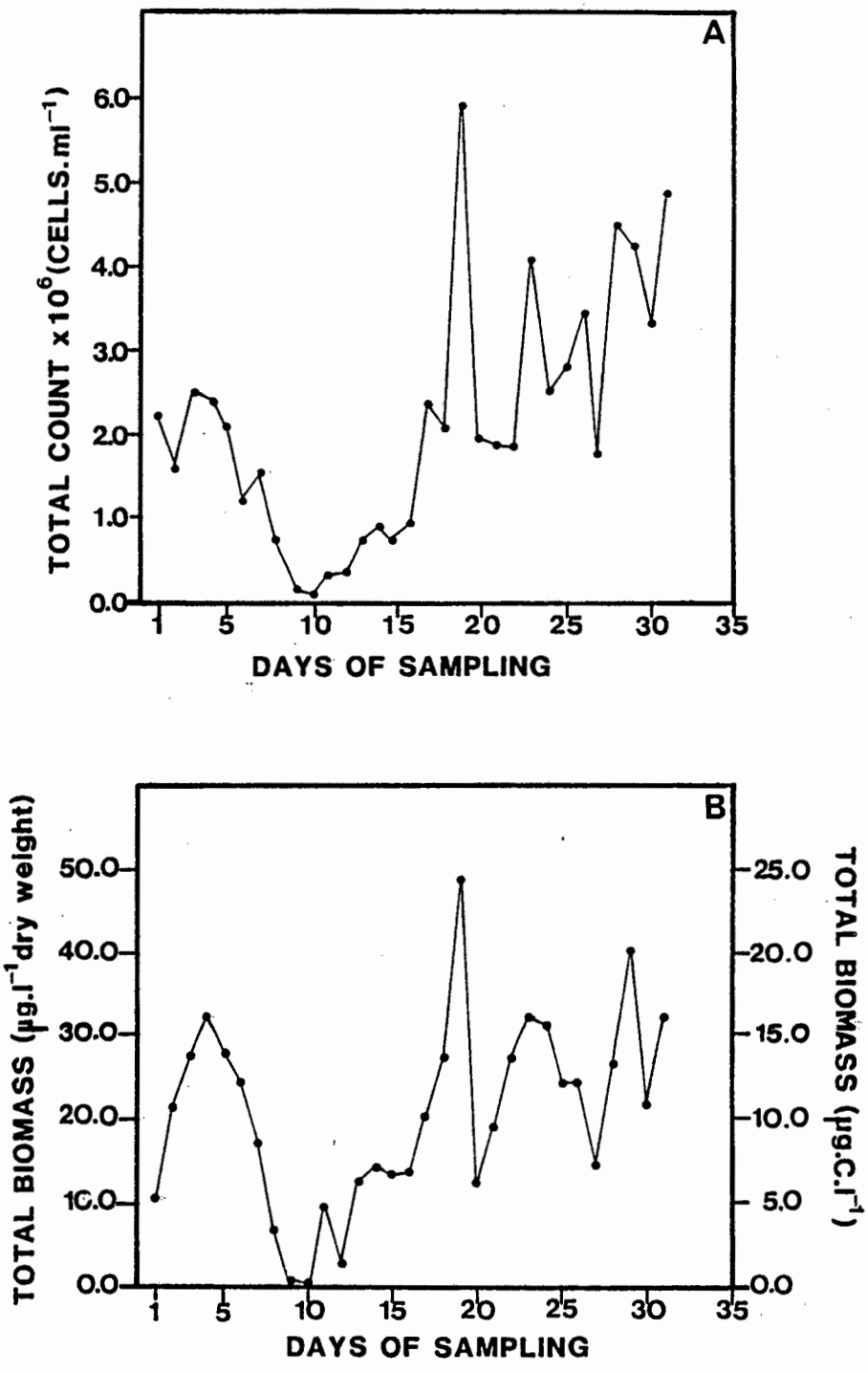

FIGURE 9: Total bacterial numbers (cells x 10^6.ml^{-1}) and biomass (µg.l^{-1} dry weight and µg bacterial C.l^{-1}) at Oudekraal during the winter sampling period. (Fig 9a: Total count. Fig. 9b: Total biomass).

attributable to upwelling.

2.3.5 Bacterial biomass

2.3.5.1 Summer

Results of biomass determinations for the summer sampling period are presented in fig.8b. The mean recorded biomass was 23.88µg $C.l^{-1}$ but there was marked variation in the values recorded (s = 17.05µg $C.l^{-1}$) which was strongly associated with the occurrence of up and downwelling. A strong positive correlation was observed between water temperature and total biomass, and strong positive correlations were noted between total biomass and the nitrogen ratios $NO_3 + NO_2$/Total N and $NO_3 + NO_2/NH_4^+$ + urea (Table 1a). Total biomass was observed to fall to low levels of <10.0µg $C.l^{-1}$ during upwelling (water temperature <11°C) and to rise to levels >50.0µg $C.l^{-1}$ during downwelling. As the biomass was strongly correlated with hydrological conditions, rapid changes in biomass were observed to occur from day to day, following changes in wind direction.

2.3.5.2 Winter

Results of biomass determinations for the winter sampling period are presented in fig.9b. The mean

recorded biomass during winter was lower than that recorded during summer (mean = 10.38µg $C.l^{-1}$) but the range of values recorded was less (s = 5.86µg $C.l^{-1}$) While it was evident that the occurrence of upwelling induced a drop in bacterial biomass on days 8, 9 and 10 of sampling, no correlation was present between total biomass and water temperature, nor between biomass and the nitrogen ratios NO_2 + NO_3/Total N and NO_3 + NO_2/NH_4^+ + urea (Table 1b).

2.3.6 Viability and percentage plateability

2.3.6.1 Summer

Viable counts (V.C.) and percentage plateabilities for the summer sampling period are presented in figs 10a and b. With rare exceptions, both the V.C. and %P recorded on 0.1% Pep-SWA were higher than those on 0.5% Pep-SWA. The results of correlation analyses showed that there was a weak negative correlation between temperature and the V.C. and %P, and a positive correlation between the nitrogen ratios (NO_3+NO_2 / NH_4^++ urea ; NO_3 + NO_2 / Total N) and both the V.C. and %P. This appears to be paradoxical, since there was a marked coincident drop in both the C.F.U. count and %P with the onset of upwelling, assessed both by water temperature and the nutrient

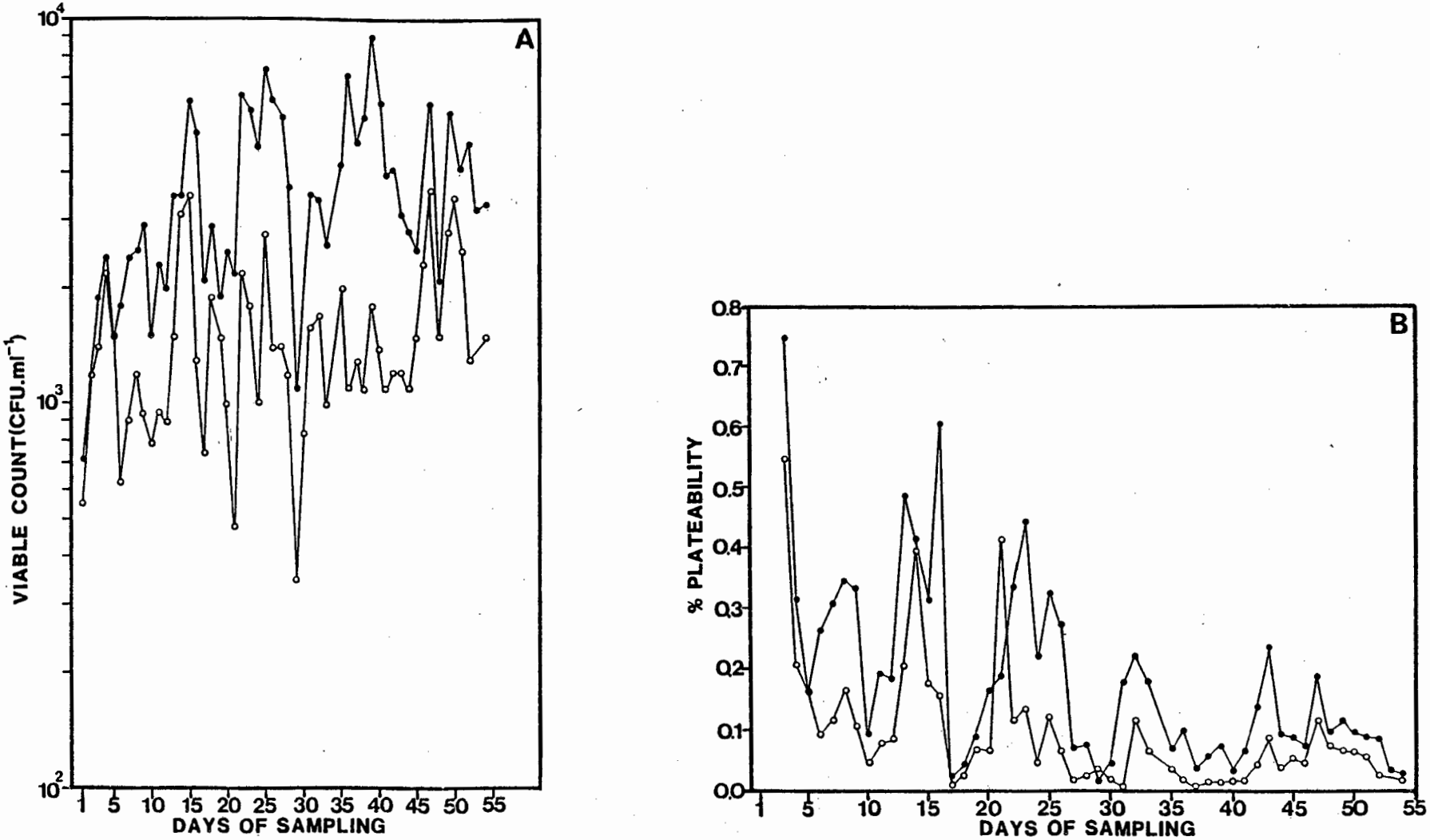

FIGURE 10: Viable count (CFU.ml^{-1}) and percentage plateability of bacterial populations at Oudekraal, determined from 0.1% Pep-SWA (—●—) and 0.5% Pep-SWA (—o—). (Fig. 10a: Viable count - Summer. Fig. 10b: % Plateability - summer).

status of the water (figs 3, 4, 5). This can be explained by reference to fig.11 which gives the mean C.F.U. count for each recorded temperature, which suggests that, although the C.F.U. count at temperatures <11°C are substantially lower than those at higher temperatures, there is an inherent bias introduced by the rarity of data from extremes of the temperature range and by a slight decrease in the V.C. at the highest temperature recorded. Plateabilities were also variable under seemingly constant hydrological conditions: there appeared to be a temporal sequence in the plateability in which both the V.C. and %P were reduced immediately after upwelling commenced, increased rapidly once upwelling had ceased, but dropped again before the following upwelling, particularly if the intervening period was >3 days.

2.3.6.2 Winter

Viable counts and percentage plateabilities for the winter sampling period are presented in figs 10c and d. As in the summer period, the V.C. and %P on 0.1% Pep-SWA were generally higher than those recorded on 0.5% Pep-SWA. Throughout winter, the V.C. was very similar to that recorded in summer, but as the total count (fig.9a) was substantially lower, the %P calculated was higher. The correlations between all upwelling indices

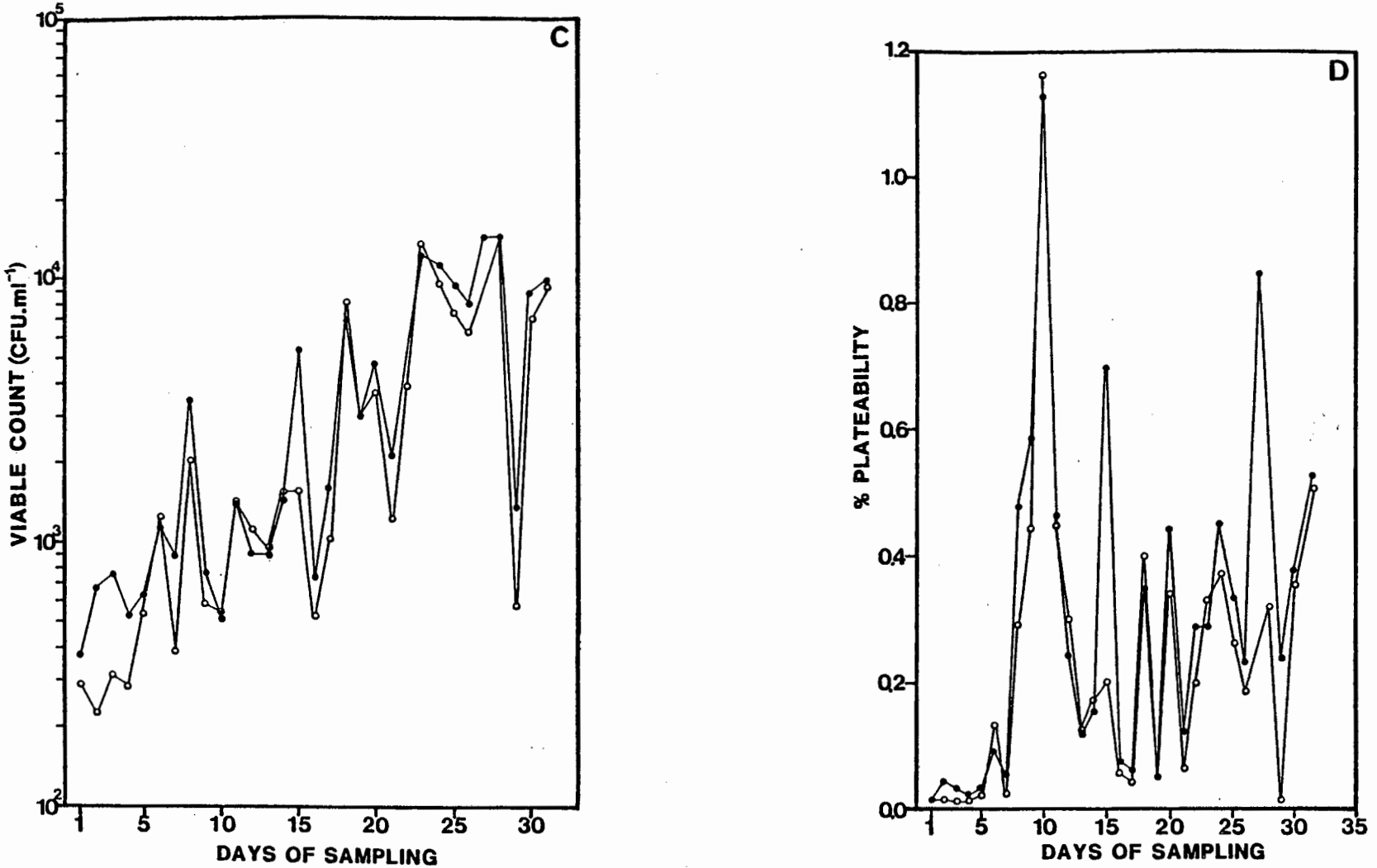

FIGURE 10: Viable count (CFU.ml^{-1}) and percentage plateability of bacterial populations at Oudekraal, determined from 0.1% Pep-SWA (—●—) and 0.5% Pep-SW (—o—) (Fig. 10c: Viable count - winter. Fig. 10d: % Plateability - winter).

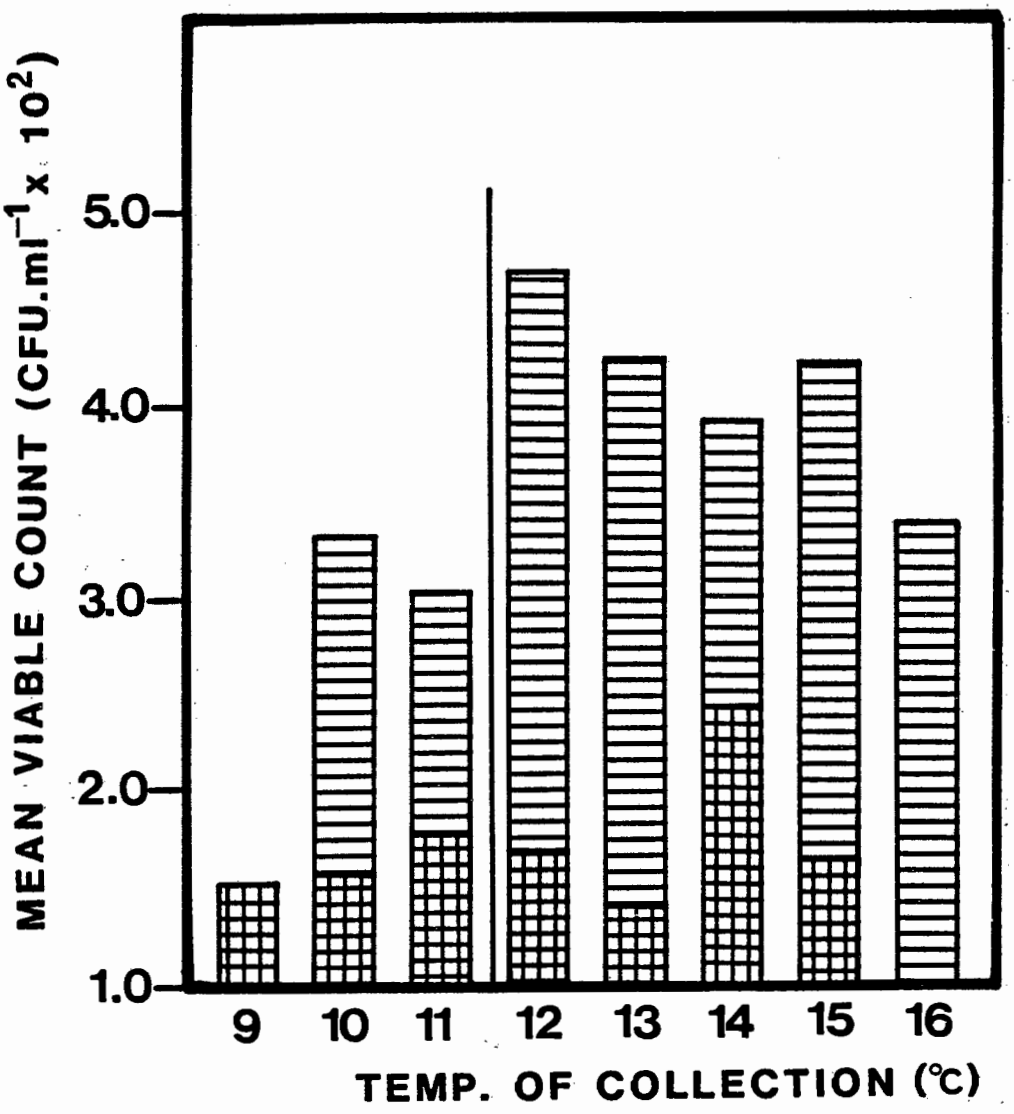

FIGURE 11: Mean viable count (CFU.ml^{-1} x 10^2) at various water temperatures during Summer, recorded on 0.1% Pep-SWA ▤ and 0.5% Pep-SWA (▦).

and the V.C. and %P were very weak, and none were significant at the 1% level, but it was noted that the upwelling which occurred on day 9 of sampling resulted in a marked drop in the V.C. This was not reflected in the %P however, since the total count fell to very low levels at this time, resulting in an apparent increase in the %P. For the duration of the winter sampling, both the V.C. and %P showed marked fluctuations from day to day.

2.3.7 Generic composition

2.3.7.1 Summer

Results of determinations of generic composition for the plateable population on days 18, 20, 23, 25, 31, 33, 35, 37 and 38 are presented in fig.12a. Populations on each of these days were characteristically diverse, and there was little to distinguish populations derived from upwelling or warm waters: variation amongst populations derived from days of upwelling (days 20, 23, 31, 33) or downwelling and warm waters appears as great as the variation between them. A striking feature of the summer population was the predominance of facultative anaerobes: only on days 23 and 38 did they comprise less than 50.0% of the population.

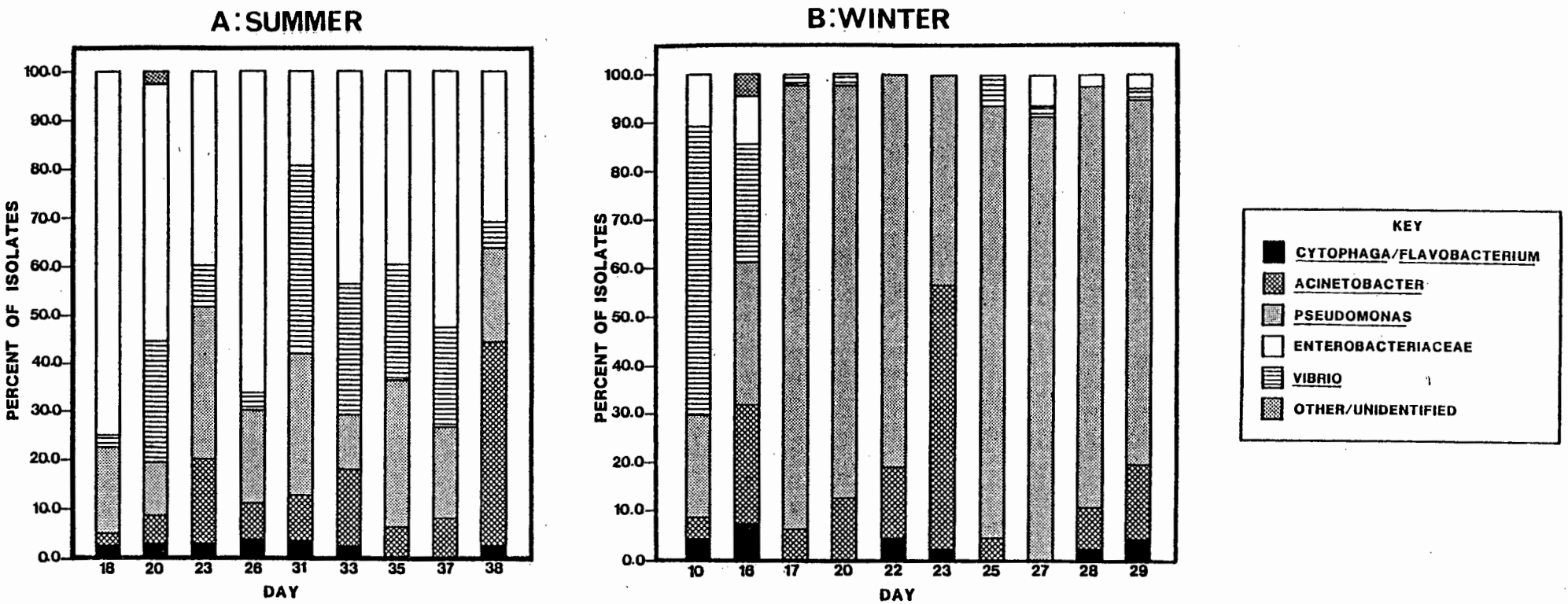

FIGURE 12: Generic composition of the plateable populations for selected sampling days. (Fig. 12a: Summer sampling period. Fig. 12b: Winter sampling period.)

2.3.7.2 Winter

Results of determinations of generic composition for the plateable winter populations of days 10, 16, 17, 20, 22, 23, 25, 27, 28 and 29 are shown in fig.12b. The two initial populations (days 10, 16) were very similar in composition to those found in the summer population, comprising a diverse assemblage dominated by facultative anaerobes (Vibrio and Enterobacteriaceae). By day 16, although still diverse, the population was dominated by oxidative strains (Pseudomonas, Acinetobacter and Cytophaga/Flavobacterium). From day 17 onwards, however, and persisting till day 23, the population was entirely composed of oxidative strains, (Pseudomonas, Acinetobacter, Cytophaga/Flavobacterium) and only in the later stages of sampling (days 25, 27, 28 and 29) were facultative anaerobes again observed, and then in very small numbers.

2.3.8 Multivariate Analysis

2.3.8.1 Strain composition of individual days

Classification and ordination analyses were performed on plateable isolates from days 18, 20, 33, 35 and 37 of the summer sampling and days 10, 22, 23, 27 and 28 of the winter sampling. Tables summarising the results of these

Table 2: Summary data matrix of percentage of isolates showing a positive reaction to discriminatory tests: summer sampling period.

Test	DAYS OF SAMPLING 18	20	23	26	31	33	35	37	38
Chromogenic	6.8	1.5	1.3	2.2	2.0	1.3	6.1	13.7	10.4
Coccoid	2.5	5.6	2.9	0.0	12.9	4.5	9.1	0.0	0.0
Motile	92.5	69.4	80.0	85.2	48.4	52.3	69.7	71.1	51.3
Caseinase +	22.5	22.2	14.3	14.8	16.1	22.7	48.5	10.5	15.4
Caseinase ++	15.0	2.8	11.4	3.7	9.7	4.5	15.2	2.6	5.1
Gelatin liq +	85.0	63.9	40.0	70.3	41.9	47.7	63.6	56.0	59.0
Gelatin liq ++	35.0	36.1	14.3	44.4	19.4	29.5	24.2	39.5	38.5
Agarase +	7.5	16.7	17.1	14.8	16.1	11.4	18.2	13.2	2.6
Agarase ++	7.5	13.9	5.7	7.4	0.0	9.1	9.1	2.6	0.0
C.M.C^{ase} +	40.0	5.6	0.0	11.1	19.4	11.4	33.3	10.5	2.6
C.M.^{Case} ++	7.5	5.6	0.0	3.7	9.7	4.5	24.2	7.9	2.6
Oxidase +	67.5	61.1	8.6	96.2	45.2	84.1	81.8	68.9	66.7
Catalase +	65.0	83.3	22.9	63.0	58.1	63.6	54.5	26.3	10.3
Catalase ++	15.0	13.9	8.6	7.4	29.0	52.3	36.4	0.0	7.7
Oxidative-glucose	72.5	77.8	45.8	61.3	77.0	79.5	69.7	76.3	41.0
Oxidative-mannitol	47.5	75.0	37.1	53.6	54.8	72.7	57.6	57.9	43.6
Fermentative-glucose	70.0	77.8	45.7	64.3	67.7	77.3	63.6	73.7	43.6
Fermentative-mannitol	55.0	72.2	31.4	53.6	54.8	65.9	51.5	57.9	33.3
NO_3 reductase +	65.0	97.2	60.0	74.1	67.7	84.1	66.7	63.2	71.8
NO_3 reductase ++	47.5	86.1	57.1	67.7	61.3	77.3	60.6	57.9	61.5
NO_2 reductase	0.0	0.0	0.0	3.7	0.0	0.0	6.1	2.6	5.1
Growth at 30°C +	97.5	91.7	51.4	88.9	90.3	75.0	90.9	78.9	64.1
Grwoth at 30°C ++	82.3	75.0	22.9	14.8	32.3	9.1	72.7	39.5	25.6
Growth at 37°C +	65.0	69.4	0.0	3.7	19.4	4.5	18.2	5.3	2.6
Growth at 37°C ++	12.5	5.6	0.0	0.0	0.0	0.0	9.1	0.0	0.0
Growth on Cetrimid-SWA	20.0	11.1	8.6	37.0	0.0	4.5	21.2	34.2	33.3
Growth on T.C.B.S.	30.0	52.8	17.1	51.9	19.4	45.5	30.3	65.8	38.5
n =	40	36	35	27	31	44	33	38	39

Table 3: Summary data matrix of percentage of isolates showing a positive reaction to discriminatory tests: winter sampling period.

Test	DAYS OF SAMPLING 10	16	17	20	22	23	25	27	28	29
Chromogenic	4.3	12.2	0.0	0.0	6.4	2.1	0.0	0.0	2.2	6.7
Coccoid	0.0	4.9	0.0	0.0	2.1	27.1	4.3	0.0	0.0	2.2
Motile	36.2	48.8	89.6	100.0	83.0	45.8	89.4	97.9	91.3	82.2
Caseinase +	19.1	41.5	64.6	63.8	27.7	16.7	83.0	75.0	47.8	0.0
Caseinase ++	2.5	2.4	6.3	51.1	4.3	0.0	72.3	16.7	17.4	0.0
Gelatin liq +	59.6	61.0	93.8	100.0	85.1	50.0	83.0	87.5	87.0	55.6
Gelatin liq ++	31.9	34.1	62.5	61.7	34.0	33.3	74.5	77.1	63.0	6.7
Agarase +	44.7	29.3	39.6	31.9	44.7	2.1	44.7	68.8	63.0	77.8
Agarase ++	21.3	22.0	18.8	10.6	8.5	2.1	36.2	60.4	56.5	4.4
C.M.Case +	27.7	14.6	22.9	36.2	10.6	10.4	8.5	20.8	15.2	6.7
C.M.Case ++	6.4	9.8	16.7	27.7	8.5	8.3	4.3	16.7	13.0	4.4
Oxidase +	57.4	63.4	100.0	100.0	89.4	97.9	97.9	91.7	95.6	64.4
Catalase +	50.0	45.0	45.0	40.0	36.2	33.3	19.1	41.7	8.7	4.4
Catalase ++	5.0	4.0	4.0	2.0	4.3	6.3	2.1	2.1	2.2	0.0
Oxidative-glucose	40.4	41.5	6.3	0.0	10.6	43.8	4.3	2.1	0.0	82.2
Oxidative-mannitol	68.1	24.4	2.1	0.0	0.0	0.0	4.3	8.3	0.0	53.3
Fermentative-glucose	42.6	29.3	2.1	0.0	0.0	0.0	2.1	2.1	0.0	4.4
Fermentative-mannitol	40.4	26.8	2.1	2.1	0.0	0.0	2.1	8.3	0.0	4.4
NO_3 reductase +	42.6	61.0	18.8	12.8	23.4	43.8	10.6	29.2	2.2	88.9
NO_3 reductase ++	36.2	43.9	2.1	0.0	4.3	0.0	2.1	2.1	0.0	6.7
NO_2 reductase	0.0	4.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Growth at 30°C +	70.2	65.9	100.0	100.0	97.9	97.9	100.0	100.0	100.0	100.0
Growth at 30°C ++	66.0	58.5	100.0	100.0	97.9	97.9	100.0	100.0	100.0	71.1
Growth at 37°C +	21.3	43.9	83.3	93.6	93.6	66.7	85.1	85.4	56.5	11.1
Growth at 37°C ++	8.5	36.6	60.4	51.1	68.1	0.0	66.0	43.8	43.5	0.0
Growth on Cetrimid-SWA	21.3	19.5	60.4	51.1	19.1	35.4	85.1	66.7	69.6	11.1
Growth on T.C.B.S.	27.7	22.0	2.1	0.0	0.0	0.0	2.1	2.1	0.0	2.2
n =	47	41	48	47	48	48	47	48	46	45

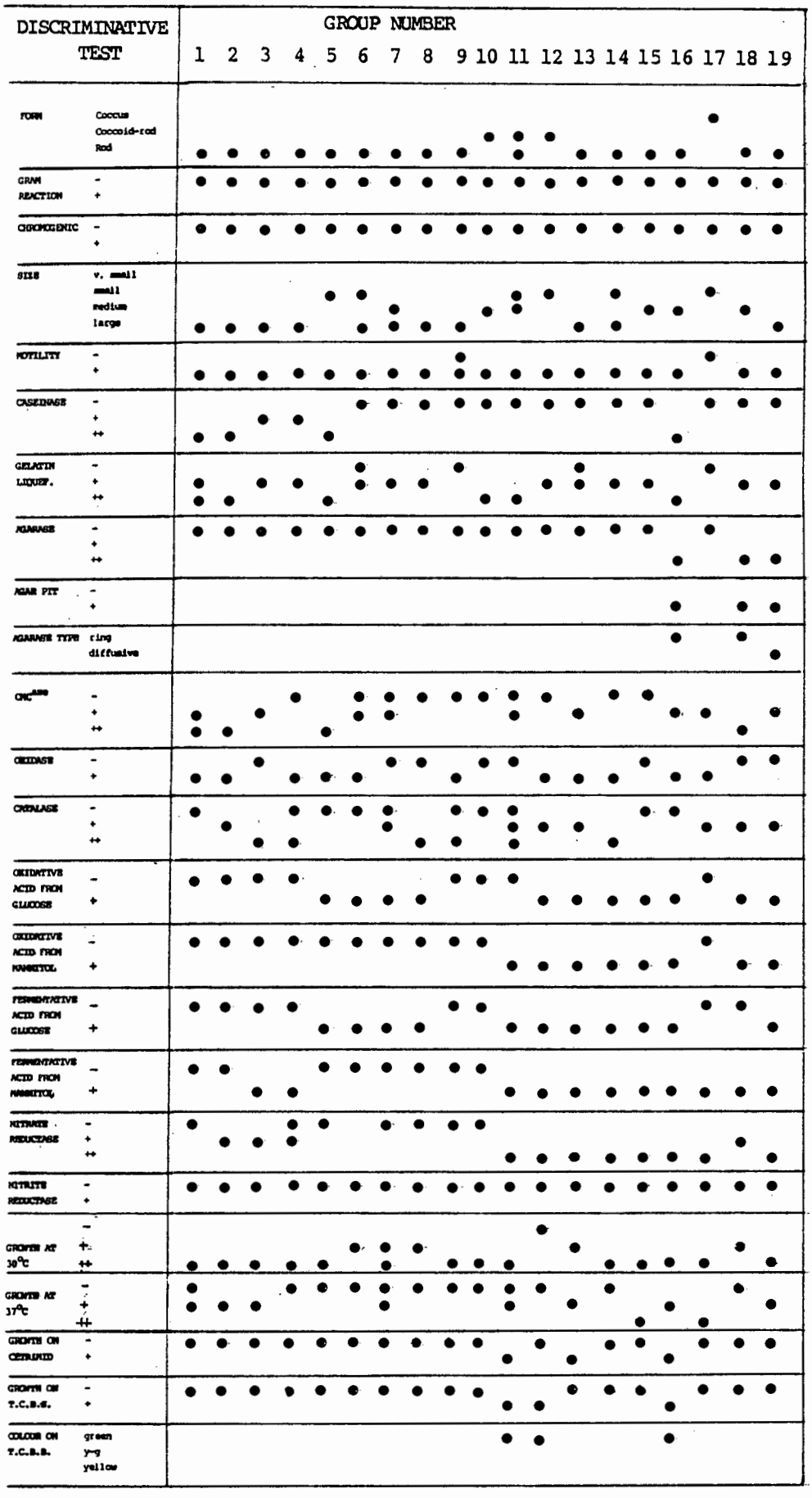

Table 4: Characteristics of groups present on day 18 of the summer sampling period, defined at the 85% S level by classification according to the Bray-Curtis measure of similarity.(● Denotes at least one member of the group showing a positive reaction to the test indicated.)

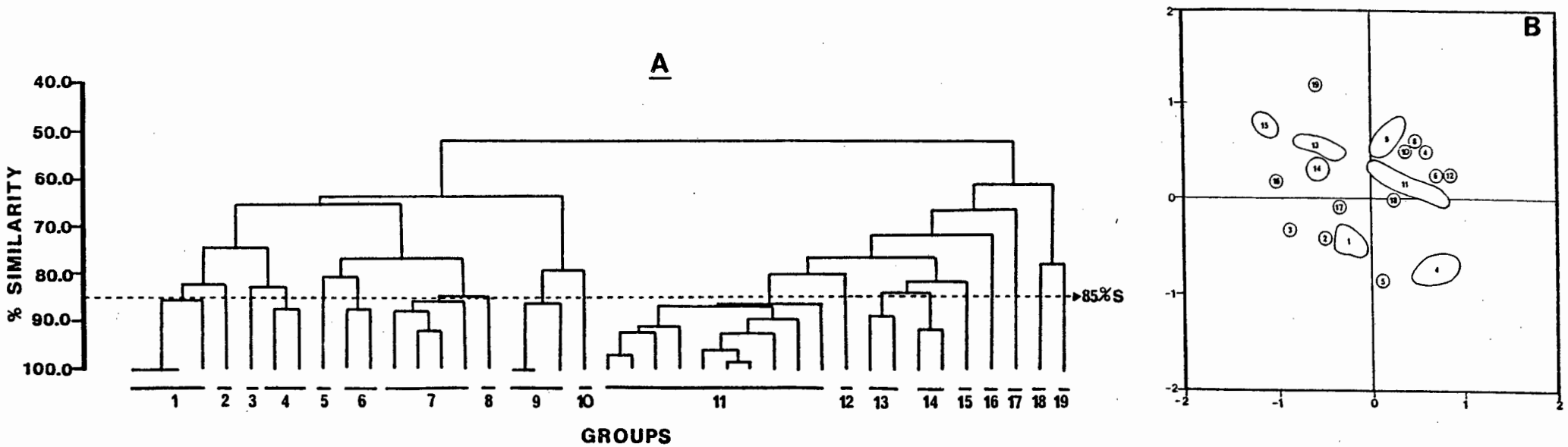

FIGURE 13: Results of similarity analysis of the randomly selected plateable population on day 18 of the summer sampling period. (Fig. 13a: Classification dendrogram. Fig. 13b: Multi-dimensional scaling ordination plot.)

discriminatory tests are presented (Tables 2, 3).

Throughout, there was good correlation with the generic groupings at the 60%S - 70%S level, but physiological groupings were defined at the 85%S level, and showed that there were a wide variety of strains present within the broader generic groupings. While it is difficult to assign specific characteristics to each of these groups, since the group presentations represent a summary of overall similarities according to the Bray-Curtis measure, a table of properties for the population of day 18 of the summer sampling is presented (Table 4) as an example of the discrimination process, together with the classification and multi-dimensional scaling ordination diagrams for day 18 (figs 13a, b). These show that there is, in general, a clear separation on the basis of physiological response, with only occasional overlaps of groups, which result largely from discriminations arising on the basis of degree of response to the tests.

Table 5 shows the number of groups defined at the 85%S level for each of the days tested.

There is little to distinguish upwelling and downwelling or warm water populations in summer - these populations all showed a high degree of group diversity. Populations tested in winter showed a much lower group diversity. An exc- eption to this was the population tested from day 10 in winter which had a large number of physiological groups present.

	SAMPLING DAY	No. of Groups (N)	n
SUMMER	18 ↓	18	40
	20 ↑	16	36
	33 ↑	20	43
	35 ↓	24	33
	38 ↓	14	39
WINTER	10 ↑	25	48
	22 ↓	11	47
	23 ↓	7	48
	25 ↓	14	48
	28 ↓	8	46

(↑= days of upwelling, ↓= days of downwelling)

Table 5: Number of physiological groups, defined at the 85%S level using the Bray-Curtis measure of similarity, for 5 selected days during summer and winter.

2.3.8.2 Summary classifications

Results of the classification of the summary data matrices (Tables 2, 3) for all days tested during summer and winter are presented in figs 14a and b.

During summer (fig.14a) little difference could be discerned between populations from upwelling or downwelling water, and all populations tested had a similarity >70.0%. While populations from days 20 and 33 (upwelling) grouped together at 85.0%S, and populations from downwelling water (days 35 and 18, days 37 and 26) also paired together, such pairings were indiscriminately grouped together, and at least one grouping of an upwelling population (day 23) and downwelling population (day 38) occurred at the 76.0%S level.

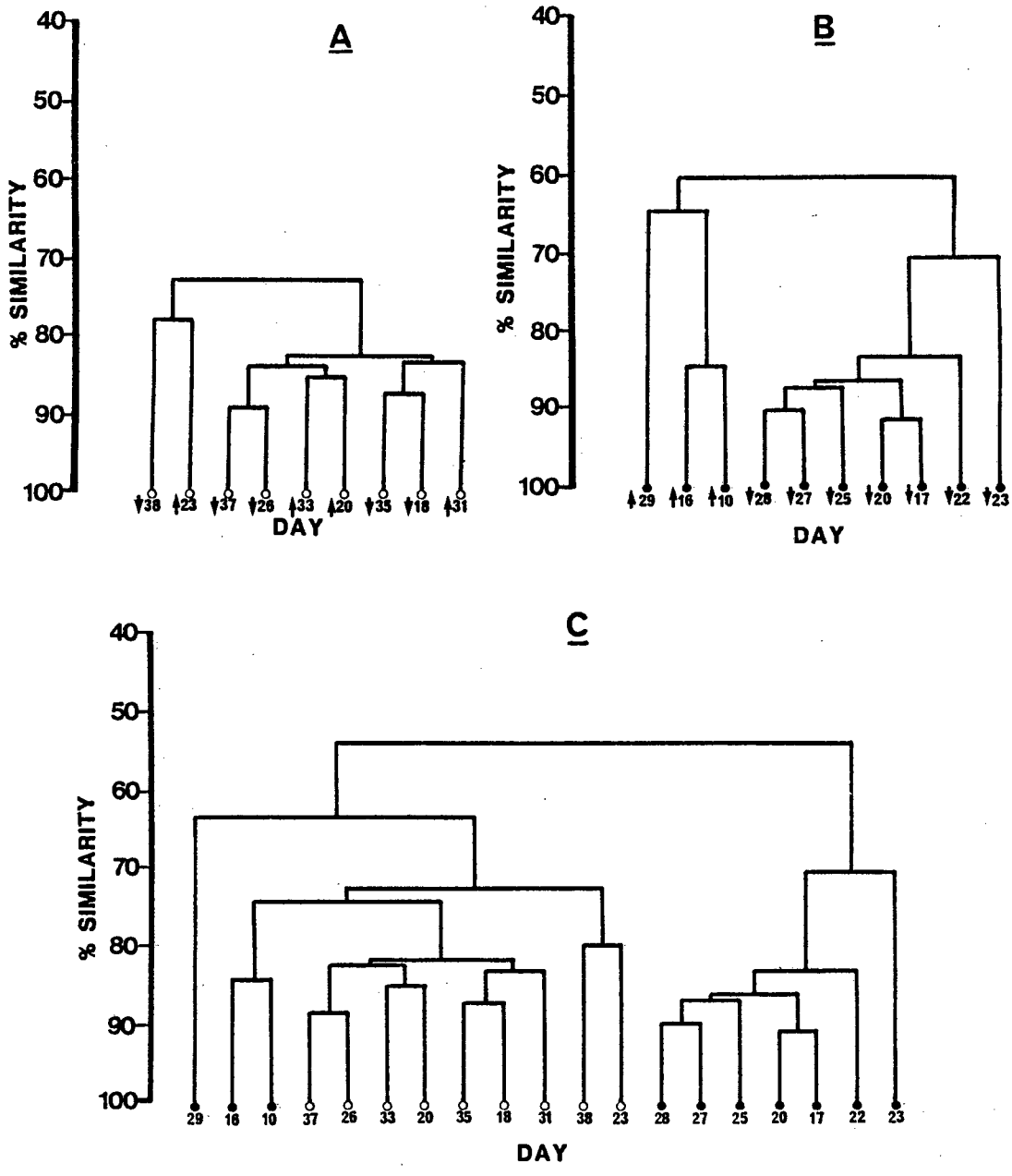

FIGURE 14: Results of similarity analysis for each of the days on which bacterial population characteristics were determined, based on summary data matrices (Tables 3, 4). (Fig. 14a: Summer sampling period (○). Fig. 14b: Winter sampling period (●) Fig. 14c: Combined data matrices. ↑ = upwelling conditions. ↓ = downwelling conditions).

During winter, however, populations sampled close to an upwelling event (days 10,16 and 29) were clearly distinguished from those sampled during downwelling (days 17, 20, 22, 23, 25, 27, 28) at the 60%S level (fig.14b). Even though the population sampled on day 29 was derived from weakly upwelled, mixed water (fig.2b) it nonetheless still grouped with the populations derived from profound upwelling on days 10 and 26, at the 63.4%S level.

When the two data matrices were combined (fig.14c) two groups were defined at the 54.1%S level. The first consisted of a grouping of the summer populations and the winter upwelling populations from days 10 and 16, defined at the 74.0%S level, together with the population from day 29 (winter), which was grouped at the 62.4%S level. The second group was entirely composed of populations derived from winter downwelling waters, grouped at the 70.0%S level.

2.3.9 Diversity

Two indices of diversity were calculated, based on the classification dendrograms (Table 5), using groups defined at the 85%S level. The first (fig.15a, c) is a simple calculation of the number of groups present at the 85%S level as a percentage of the total number of groups possible (which = n). The second is the Shannon-Weaver index of diversity (H') which takes into account the proportion of n which occupies each group (fig.b,d).

During summer, the Shannon-Weaver indices remained high (H'>3.0) throughout, with only slight variations apparent between upwelling and downwelling. The highest value of H' recorded occurred on day 35 of sampling during the onset of downwelling after a long (4 day) preceding period of upwelling. Values of D confirmed the greater diversity of the population on this day, and the subsequent drop in diversity as downwelling continued to day 38.

During winter, with the exception of the population tested on day 10 of sampling, both diversity indices

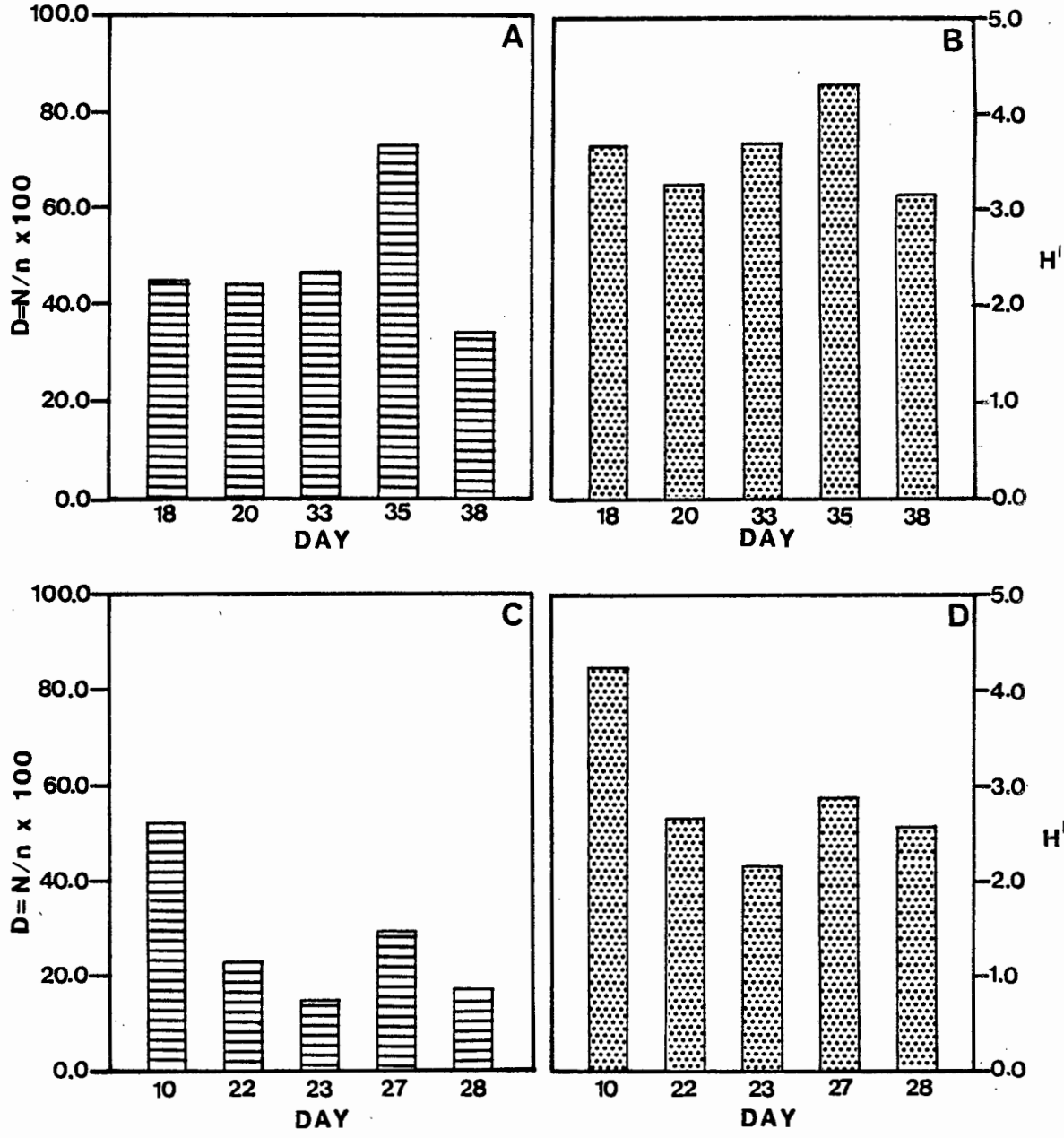

FIGURE 15: Statistics of diversity (Shannon-Weaver Index (H') - ▨, $D = {}^{n}/_{N} \times 100$ - ▤) for 5 selected days during summer and winter. (Fig. 15a, b: Summer sampling period. Fig. 15c, d: Winter sampling period).

remained low (D<30.0%, H'<3.0). The diversity of the population tested on day 10 was high (D = 52.08%, H' = 4.22) and hence similar to the diversities recorded during summer.

2.4 Discussion

On the basis of temperature and nutrient analyses, sampled at a fixed shore station, it was shown that wide fluctuations in hydrological conditions took place and that these fluctuations encompassed both day to day variations as well as periodic cyclical changes extending over lengthier periods. During summer (fig 3a) such cycles of increasing and decreasing temperature and nutrients occurred rapidly (3 - 5 days) and with marked regularity. So, for example, it was noted that in response to persistent offshore south-easterly winds, upwelling of South Atlantic Central Water occurred at the coast which resulted in rapid decreases in water temperature, which correlated closely with increases in the NO_3, NO_2 and total nitrogen content of the water. Such phenomena have been extensively investigated by Andrews and Hutchings (1980), Andrews and Cram (1969) and Jury *et al*. (1985a, b).

After relaxation of offshore winds, rapid warming of surface waters was observed to occur: in some instances,

very light or absent winds resulted in the maintenance of recently upwelled water local to its point of origin, but the onset of onshore winds was observed to move warm waters back inshore in downwelling events which resulted in the return of offshore waters of upwelled origin.

While upwelled waters were observed to have high NO_3 and NO_2 concentrations, once upwelling had ceased NO_3 concentrations were observed to fall rapidly (figs 4,5). In addition, while newly upwelled water had a low Chl a content (P. Fielding pers. comm.), once upwelling had ceased, the Chl a content was observed to increase rapidly and further increases were observed if downwelling occurred.

This is strongly suggestive of a rapid colonization of newly upwelled water by phytoplankton cells, and is supported by the observed decrease in nutrient levels once upwelling had ceased. Barlow (1982b), studying a single patch of upwelled water by means of a drogue, showed that as newly upwelled, nutrient rich water is exposed to light and seeding with phytoplankton cells, dense phytoplankton blooms develop rapidly (within 3 - 5 days), and result in a decrease in the levels of NO_3, SiO_3 and PO_4. Such rapid growth is characteristic of upwelling areas when compared with the slower rates

recorded in temperate waters, where such blooms may take weeks rather than days to develop (Morris and Skea, 1978). Barlow (1982a, b) suggests that both light and nutrient levels are the critical determinants in production and maintenance of the phytoplankton bloom, and that NO_3 levels <1µg at.l^{-1} are limiting to phytoplankton growth (Barlow, 1984).

During the summer sampling period the NO_3 concentration regularly approached limiting levels, but it was constantly renewed by subsequent upwellings, which would maintain high levels of phytoplankton activity in the nearshore region. As pointed out by Brown and Field (1986) however, a principal determinant in the development of phytoplankton biomass is the size of the initial seeding stock, which is in turn dependent on the extent of mixing of newly upwelled and aged waters: as a result, both nutrient and Chl a levels were observed to vary considerably during inter-upwelling periods, and appear to be influenced by the profundity of the preceding upwelling (fig.4 and P. Fielding, pers. comm.).

The size of the bacterial population was also observed to vary through both the summer and winter sampling periods: in both cases, the occurrence of upwelling resulted in rapid decreases in both numbers and biomass.

Once upwelling ceased, however, numbers and biomass of bacteria were observed to increase, reaching very high levels during downwelling events. It was, however, observed that during extended inter-upwelling periods, and during extended downwelling, a second decrease in both numbers and biomass occurred (fig.8a, b). During summer, increases in numbers and biomass of bacteria were rapid and closely correlated with changes in the nutrient concentrations measured. So, for example, there was a strong negative correlation between bacterial numbers and biomass and the concentration of NO_3 and NO_2 (whether as a percentage of the total N or as a ratio to NH_4 + urea), which shows that during summer, both numbers and biomass of bacteria are strongly linked to the development of phytoplankton blooms, since it is the phytoplankton blooms which are primarily responsible for decreases in nutrient levels (Barlow, 1982a, b, 1984; Brown, 1981; Hutchings et al., 1984).

Such associations between phytoplankton growth and bacteria are well documented. Phytoplankton have been shown to release between 5% and 50% of fixed carbon as dissolved exudates, which are utilised by the micro-heterotrophic community (Thomas, 1971; Itturiaga and Hoppe, 1977; Lancelot, 1979; Larsson and Hagstrom, 1979, 1982; Cole et al., 1982) and it can be

hypothesised that phytoplankton bloom development is the principal determinant of bacterial growth and activity in this system.

Marked seasonal differences were observed to occur between the summer and winter samplings. Whereas, during summer, there were very rapid alternations between offshore and onshore winds, upwelling and downwelling events, with associated changes in nutrients, Chl a and bacterial numbers and biomass, conditions in winter appeared more constant. Upwelling events in winter were rare and of short duration, interspersed with long periods of onshore winds which did, however, vary considerably in strength (fig.3b). As a result of the rarity of upwelling, the mean water temperature during winter (15.5°C s = 1.01°C) was higher than that recorded during summer (12.1°C s = 1.66°C) but the NO_3 concentration was in general lower (winter NO_3 mean = 6.94 µg at NO_3-N.1^{-1} summer NO_3 mean = 9.3 µg at NO_3-N.1^{-1}). Owing to the increased phytoplankton activity in summer, however, NO_3 levels regularly approached limiting levels, while those in winter were always >2.0 µg at NO_3-N.1^{-1}. The infrequent winter upwellings led to pronounced increases in the NO_3 concentration (fig.5a) but the dissipation of

these high concentrations was felt to be largely due to mixing with surrounding lower nutrient waters, since Chl a levels were low and bloom development subsequent to upwelling was slow, owing to the low ambient light levels (P. Fielding, pers. comm.) In addition, augmentation of nutrient levels resulted from the onset of winter storms: increases in both NO_3 and NO_2 concentrations were noted when such storms occurred and were probably the result of liberation of nutrients from benthic sediments.

Mazure (1978, 1980) recorded a seasonal cycle of bacterial numbers and biomass at the same study site, in the water column and associated with kelp fronds, although sampling in that study was conducted on a monthly and not daily basis. In that study, a numbers and biomass minimum was shown to occur during winter, with rapid increases occurring during the summer upwelling season, these changes being closely correlated with phytoplankton standing stocks and macro-algal production (Mazure, 1978). In the present study, the numbers of bacteria present during winter were shown to be lower than those present during summer (summer mean = 3.91×10^6 cells.mℓ$^{-1}$s = 3.70×10^6 cells.mℓ$^{-1}$, winter mean = 2.16×10^6 cells.mℓ$^{-1}$ s = 1.52×10^6 cells.mℓ$^{-1}$) but the variation is not as great as that shown by Mazure

(1978). Bacterial biomass was also shown to vary between summer (mean = 23.88µg $C.l^{-1}$ d.w. s = 17.05µg $C.l^{-1}$) and winter (mean = 10.38µg $C.l^{-1}$. s = 5.86µg $C.l^{-1}$) which were both, however, substantially more than those recorded by Mazure (1978).

Decreases in the numbers and biomass of bacteria occurred during the winter upwelling episodes, but the subsequent development of the bacterial population was slower than that which occurred during summer. It is felt that this was the result of the slow development of phytoplankton populations in winter upwelled water (fig.9a, b). In the absence of a refined assessment of bacterial activity it was hoped that bacterial plateabilities would provide an index of the nutritional capabilities of the population (Poindexter, 1981; Torrella and Morita, 1982; Amy et al., 1983; Amy and Morita, 1983a; Kurath and Morita, 1983; Laake et al., 1983a). With rare exceptions, viable counts and %P were higher on lower nutrient (0.1% Pep-SWA) plates.

This suggests that an inability to form colonies is a reflection of the lack of mechanisms competent to deal with high nutrient levels, which results from the prolonged starvation to which cells in newly upwelled or nutrient depleted aged water have been subjected

(Novitsky and Morita, 1977, 1978; Amy et al., 1983). During summer, it was observed that both the viable count and %P fell with the onset of upwelling. Recovery of plateability was rapid however, but no reliable correlation could be drawn with subsequent population development. It is suggested that the mechanisms governing plateability are complex, representing a combination of parameters largely influenced by the availability of nutrients: the data presented in summer (figs 10a, b) suggest a rapid increase in plateability after upwelling, with a subsequent decrease during prolonged inter-upwelling periods.

On the basis of these data, it is suggested that the observed properties of the water masses sampled at the fixed sampling station represent the characteristics of water with different surface exposure times and stages of biological development. During summer, continual episodic upwelling leads to the export of nutrient rich water. Subsequent development of phytoplankton and bacterial populations leads to the biological modification of the water, the extent of which depends on the length of time of exposure to a high light regime. In this way, prolonged upwelling will lead to the development of patches and plumes of water which, although it may mix with surrounding waters, bears characteristics largely determined by its upwelling

origin (fig.16). Since the furthest extent of such plumes have been exposed for the longest time, a horizontal section of the plume should reveal various stages of development of phytoplankton and bacterial development.

Jones (1973), Hauxhurst et al. (1981) and Fukami et al. (1985a, b) suggest that the development of bacterial populations is accompanied by changes in taxonomic structure which are a reflection of changes in physiological attributes of the population in response to environmental changes. These changes appear to be largely governed by the type and availability of nutrients, and in a phytoplankton dominated system, such changes in the bacterial population have been shown to be clearly associated with different developmental stages of the phytoplankton bloom (Martin, 1980; Martin and Bianchi, 1980). It can be hypothesised, therefore, that variability of bacterial populations sampled at a fixed sampling point is the result of observation of different stages of development of bacterial populations derived largely from the upwelled water population.

The population of upwelled water was shown to be low in numbers and biomass, but diverse in generic groupings and physiological attributes. It is suggested that this population was adapted to highly oligotrophic conditions

TIME	>5 DAYS	DAY 5	DAYS 3-4	DAYS 1-2
NO_3	$<2\mu g$ at.l^{-1} (limiting)	$>2<10\mu g$ at.l^{-1}	$>2<10\mu g$ at.l^{-1}	$>15\mu g$ at.l^{-1}
PHYTOPLANKTON	Stage 3 (Barlow,1984)-senescent. Biomass low. Chl a low.	Stage 2 (Barlow,1984)-mature. Biomass high. Chl a high.	Stage 2 (Barlow,1984)-log phase. Biomass increasing. Chl a increasing.	Stage 1 (Barlow,1984) lag phase. Biomass low. Chl a low.
BACTERIA	Biomass decreasing. Diversity increasing. Fermentative isolates re-appear. %P dropping.	Biomass high. Diversity low. Only oxidative isolates present. %P high.	Biomass increasing. Diversity initially high,then decreases. Oxidative isolates become dominant. %P increasing.	Biomass low. Diversity high. Fermentative isolates dominant. %P low.

FIGURE 16: Structure and characteristics of a hypothetical upwelling plume after >5 days of upwelling.

A: Oceanic and aged upwelled water.

B: Upwelled water patch.

C: Upwelling plume.

D: South Atlantic Central Water.

and displayed a low V.C. and %P as a result. Upwelled water was shown to be rich in nitrogen, particularly as NO_3, and on exposure to the euphotic zone rapidly developed a phytoplankton bloom (Barlow, 1982; Brown and Field, 1986). As the bloom developed, the D.O.C. levels would increase, as a result of exudation by the plankton (Thomas, 1971; Cole, 1982; Cole et al., 1982; Eberlein et al., 1983). The gradual increase in ambient D.O.C. levels led to the activation of dormant, starved cells (Amy et al., 1983; MacDonell and Hood, 1982) and uptake of D.O.C. prompted the increase in numbers and biomass of the bacterial population (Bolter, 1981; Laake et al., 1983a, b). Accompanying the growth of phytoplankton, the nutrient levels fell, approaching limiting levels (1.0µg at NO_3-$N.l^{-1}$) in downwelling water, but as the period of the upwelling-downwelling cycles was only 5 days (the length of time estimated by Barlow [1984] for phytoplankton blooms to enter late log phase), nutrients were continually renewed and both phytoplankton and bacterial populations were maintained in an early stage of development. As a result, little difference was observed in the structure of populations derived from upwelling and downwelling waters in summer, although significant differences in numbers and biomass did occur. These populations were characteristically diverse with a high proportion of facultative anaerobic isolates.

During winter, however, upwellings were rare and as light levels were reduced, phytoplankton biomass was low. Upwelling was followed by development of reduced phytoplankton blooms (P. Fielding pers. comm.) but the inter-upwelling period was sufficiently long as to allow bacterial populations to proceed to a late stage of development, and was accompanied by marked changes in the structure of the population. Although the winter upwelling population was very similar in structure to the summer populations, it was replaced by a population of low diversity, dominated by oxidative isolates. These results demonstrate that considerable short term variations in hydrological and biological parameters occur on a daily and seasonal basis in coastal waters on the west coast of South Africa. The hypothesis that these variations result from biological modification of upwelled water is experimentally tested in Section 3.

SECTION 3: CHANGES IN THE STRUCTURE AND ACTIVITY OF BACTERIAL POPULATIONS ASSOCIATED WITH PHYTOPLANKTON BLOOM DEVELOPMENT IN AN EXPERIMENTAL ENCLOSURE OF UPWELLED WATER

3.1 Introduction

The work described in Section 2 showed that rapid changes take place in the bacterial populations of the Benguela Upwelling System in response to changing hydrological conditions. It was suggested that these marine bacterial communities are highly dynamic and rapidly respond to changes in biological and physical conditions in terms of numbers, biomass and taxonomic structure. In other systems, such adaptability is well documented and such factors as temperature (Sieburth, 1967; Trentham and James, 1981) and nutrient availability (Bolter, 1977; Bolter *et al.*, 1977) have been shown to effect changes in both the structure and activity of heterotrophic populations.

As shown by Mazure (1978), Linley and Field (1982), Davis *et al.* (1983), Davis (1985) and data presented in Section 2, waters local to the West coast of South Africa have a high bacterial density with cells of varying morphotype and biomass. A great variety of taxonomic types occurs, which display diverse physiological characteristics. Work on the degradation of phytoplankton and macrophyte debris and detritus has shown the importance of the bacterial population in recycling of nutrients (Griffiths and Stenton-Dozey, 1981; Linley and Newell, 1981; Koop

et al., 1982a, b; Koop and Griffiths, 1982; Newell and Field, 1984) and is also strongly suggestive of successions in the bacterial communities involved. In none of these studies, however, were bacterial communities described beyond a simple breakdown according to morphotype and biomass, and in no local work have the taxonomic structure, metabolic activity or catabolic properties of such populations been assessed: rather, there has been a tendency to regard them as temporally and spatially homogenous units.

Two factors militate against such an approach. In the first place, much of the population at any one time may be metabolically inactive - it is thus essential to assess the overall heterotrophic activity of the bacterial population. Extensive work on starvation-survival strategies of bacteria in oligotrophic waters by Novitsky and Morita (1976, 1977, 1978) Tabor et al. (1981) Amy et al. (1983) and Kurath and Morita (1983), as well as micro-autoradiographic determinations of metabolic activity (Meyer-Reil, 1978b; Tabor and Neihof, 1982, 1984) shows that much of the population observed by direct count techniques is metabolically inactive. MacDonell and Hood (1982) however, showed that dormant strains revived under the appropriate nutrient conditions, could be assigned to the same genera as strains

which were plateable under normal conditions: Martin and Bianchi (1980) suggest that this is comparable to systems described by Margalev (1967, 1968) in phytoplankton, in which different sections of the population respond to specific environmental conditions, lapsing into dormancy as changing conditions favour another segment of the population. In this way, the whole population remains highly adaptable, capable of a rapid response to temporarily favourable conditions and an efficient utilisation of whichever resources are available in an otherwise oligotrophic environment.

Such changes in the population may be manifested as taxonomic or physiological changes, and synergistic and competitive strategies may result in the appearance of successions of taxonomic types. Jones (1973) showed that bacterial communities in bag enclosures in Blelham Tarn were characterised by successions of bacteria over a period of months, while Hauxhurst et al. (1981) showed that in the Gulf of Alaska, bacterial communities were taxonomically and physiologically diverse and that different communities had characteristics which enabled a rapid and efficient response to changing environmental conditions. Fukami et al. (1981, 1983, 1985a, b) showed that during the degradation of dead phytoplankton cells, distinctive successionary patterns occurred which could

be assessed by a classification of plateable bacteria: concommitant with changes in total number and biomass, rates of uptake of D.O.C. and nutrients, there were changes in the generic structure of the bacterial assemblage. In addition, they noted that at least two major populations occurred, the first dependent on the uptake of D.O.M. and the mineralisation of labile particulates, and the second dependent on the mineralisation of refactory particulates. Such changes in the composition of the active bacterial population may have profound effects on the rates of mineralisation and recycling of nutrients within the system (Fenchel and Jorgensen, 1977).

Associations between bacteria and phytoplankton are well documented. For example, phytoplankton in active growth phase have been shown to release a significant proportion of fixed carbon as dissolved extracellular exudates, which may form a direct food source for microheterotrophs (Itturiaga and Hoppe, 1977; Lancelot, 1979; Cole, 1982; Larsson and Hagstrom, 1979, 1982; Itturiaga, 1981; Jensen, 1983). Although estimates vary, between 5% and 50% of fixed carbon may be released by phytoplankton as extracellular exudates (Thomas, 1971; Cole, 1982) and this has been shown to be an important nutrient source for bacteria (Cole et al., 1982; Laake et al., 1983a;

Jensen, 1983; Joint and Morris, 1982). So, for example, Sorokin and Mikheev (1979) showed that 70-80% of fixed carbon ultimately passes through the microheterotrophic community in the Peruvian upwelling system, while Laake et al. (1983b) estimated that 35-40% of fixed carbon in an enclosed in situ mesocosm was incorporated by bacteria. The fraction of primary production available to bacteria depends however upon the age, physiological state and productivity of the phytoplankton bloom (Martin and Bianchi, 1980; Lancelot, 1984) and it can be hypothesised that these determinants will also affect the composition and activity of the bacterial population.

Brockmann et al. (1977, 1979) showed that during phytoplankton bloom development there were changes both in the generic composition of the algae (as described by Margalef, 1967) and in the nature of exudates produced by these algal genera. Such changes in the nature of D.O.C. may well reflect in changes in the associated bacterial assemblages, since variations in uptake ability are genetically related (Button, 1985; Davis and Robb, 1985).

The physical and hydrological changes which take place in upwelled water have been well documented (Andrews and Hutchings, 1980; Jury et al., 1985a, b; Shannon, 1985,

Shannon et al., 1985b) and the associated changes both in the phytoplankton and zooplankton communities have also been elucidated (Hutchings, 1979, 1981; Brown, 1983; Brown and Henry, 1985). The development of phytoplankton blooms is strongly associated with the upwelling of South Atlantic Central Water rich in nutrients. Blooms are initiated by introduction of this water into the euphotic zone and by its seeding with phytoplankton cells. Bloom development is preceded by a lag phase whose length is dependent on features such as rapidity of mixing (Brown, 1981) and the biomass of the initial phytoplankton stock (Brown and Field, 1986). Barlow (1984) divides bloom development into three phases: the first is characterised by low temperatures (<10°C) and high nutrients. The second is characterised by temperatures >10°C and NO_3 levels >2.0µg at NO_3-N.l^{-1} and <15µg at NO_3-N.l^{-1}, and the third represents a senescent or dormant phase of nutrient limitation where NO_3 falls below 1.0µg at NO_3-N.l^{-1}.

Studies conducted by Hutchings (1981), Hutchings et al. (1984) and Barlow (1982b), employing a controlled buoyancy drogue embedded in identified upwelled water plumes, have shown that patches of upwelled water may remain discrete for some time (>5 days), moving offshore for some distance before being dispersed by mixing with the surrounding waters or by subduction at the oceanic front.

It is tempting to regard such patches as semi-closed or batch systems, but no information on bacterial activity is available from these experiments.

Vertical and horizontal advection effects make it extremely difficult to follow changes in a single body of water. While drogue studies largely circumvent the problem of horizontal advection, they cannot account for vertical advection effects such as mixing across the pycnocline or sedimentation from water mass. As a result, enclosure experiments have become a common tool in aquatic ecosystem research, and have been used, for example, in in situ experiments to follow the development of phytoplankton blooms (Kuiper, 1977; Brockmann et al., 1981) Jahnke et al., 1983), changes in water chemistry (Grice et al, 1977; Koike et al., 1982; Eberlein et al., 1983; Kattner et al., 1983), development and structure of bacterial communities (Lack and Lund, 1974; Jones, 1973, 1977; Laake et al, 1983a) and have proved particularly useful in the construction of models of ecosystem dynamics (Gamble et al., 1977; Laake et al., 1983b) and cycling of nutrients such as nitrogen (Koike et al., 1982) and carbon (Ducklow et al., 1986). Reviews of such work have been done by Davies and Gamble (1979), Grice and Reeve (1982) and Davies (1984).

An enclosure of the water mass may itself affect the dynamics of the system: not only do such enclosures limit contact with the surrounding water mass, but they may exert surface or wall effects which tend to increase biotic activity (Ferguson et al., 1984). Kuiper et al. (1983) showed that bacterial numbers increased faster in small ($1.5m^3$) enclosures compared with larger ($30m^3$) enclosures, but showed that environmental factors exerted a far greater influence over the development of phytoplankton than bag dimensions: they suggest that the rapidity of bacterial development is attributable to the maintainance of detritus in a limited area. Menzel (1977) reported that patterns of succession were similar whether containment was in 4ℓ or 6.8×10^7ℓ enclosures, but small enclosures fail to provide spatial separations of activities (Kuiper, et al. 1983); as a result, most workers have tended to use enclosures of $>1.5m^3$ capacity (Grice and Reeve, 1982; Kuiper et al., 1983).

A disadvantage of such large enclosures is the necessity to site them in sheltered areas such as lakes, lochs or bays: as a result, it is not possible to use them on coasts, such as the Cape West Coast, which are highly turbulent systems. In this study, a laboratory based system was used to follow development of the biota associated with newly upwelled water. This has the

advantage of being easily sampled and replicated and similar systems have been used to follow bacterial degradative processes (Linley et al., 1981; Fukami et al., 1985a, b), development of phytoplankton blooms (Brockmann et al., 1977, 1979) and for the construction of models of nutrient regeneration (Sharp et al., 1982). The use of relatively small volumes has the disadvantage that wall effects become pronounced, however. Whipple (1901) noted that the viable count of freshwater increased 400 fold in 24 hours of storage in glass bottles, and similar effects have been noted for seawater (Zobell and Anderson, 1936; Zobell, 1943) and difficulties pertain to the extrapolation to the field of such laboratory based experiments (Gunkel, 1972). Ferguson et al. (1984) showed that total numbers, CFU count, cell volumes and turnover rate of amino acids in 3 litres of seawater, all increased within 16h of enclosure - this was chiefly attributed to enhanced activity of bacteria attached to the vessel walls.

To minimise these effects, the experiment described here utilised an aquarium tank of intermediate volume (63ℓ) designed by Ms. S.J. Painting, which was constructed to limit enclosure effects as far as was practical. It was hypothesised that changes in the biological communities which occur after upwelling could be simulated in such

an enclosure. Recently upwelled water was enclosed, exposed to a diurnal light regime and the resulting changes were monitored for a 43 day period.

3.2 Methods and Materials

3.2.1 Sample site and water collection

In December, 1983, during the summer upwelling season, 63ℓ of upwelled water was collected at Oudekraal, Cape Peninsula, South Africa (35°59.1'S, 17°21.5'E). The water was immediately transported to the laboratory, where it was pre-filtered (60μm mesh) and incubated in a pyramidical glass aquarium (fig.17). This aquarium had an aeration system which created a bubble screen along the sides, to limit settlement on the walls. In addition, a Teflon coated stirrer bar (300cm long) continuously swept the bottom of the tank to prevent particle sedimentation. The incubation was run for 43 days at 12-15°C under a diurnal light regime (13 hours on, 11 hours off) which approximated summer light conditions.

3.2.2 Sample collection: nutrients, bacterial numbers and biomass

Samples were taken at 12 hour intervals for the first 25

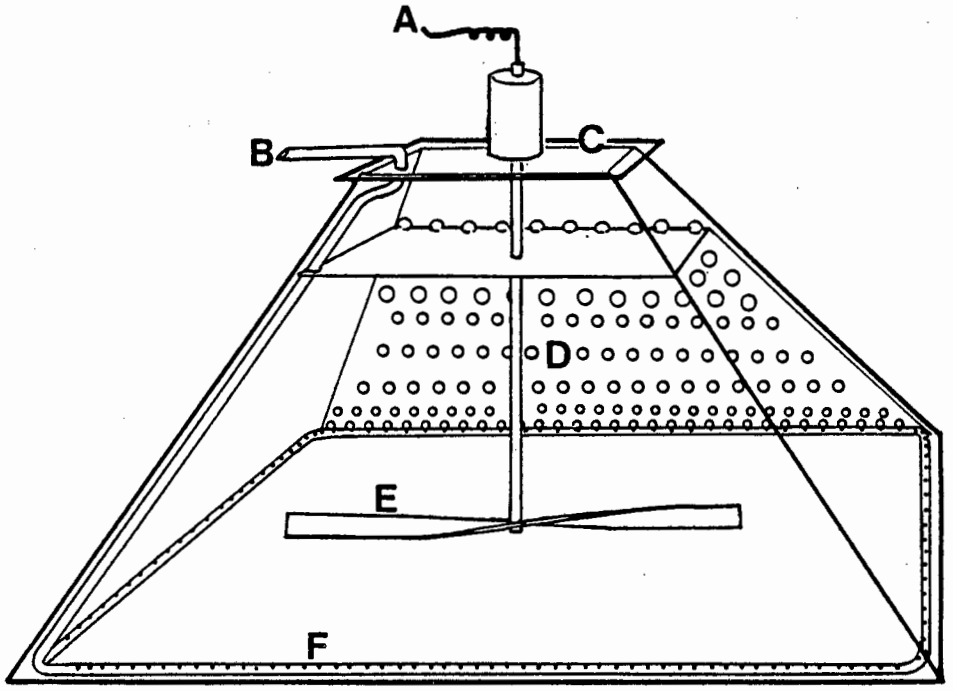

FIGURE 17: Pyramidal aquarium tank containing 63 l of newly upwelled water.

A: Motor drive to stirrer bar.

B: Air inlet to perforated aeration tube.

C: Cover.

D: Bubble screen (shown on rear wall only).

E: Teflon coated stirrer bar.

F: Perforated aeration tube.

days, and every 24 hours for the next 17 days for the measurement of NO_3, Dissolved Organic Carbon (D.O.C.), Particulate Organic Carbon (P.O.C.), bacterial numbers and biomass. Data relating to nutrients (NO_3, D.O.C., P.O.C.) appear here by permission of Ms. S.J. Painting.

3.2.2.1 NO_3 and Dissolved Organic Carbon (D.O.C.)

40ml samples were stored frozen and analysed for NO_3 and D.O.C. on a Technicon auto-analyser, following methods outlined by Mostert (1983).

3.2.2.2 Particulate Organic Carbon (P.O.C.)

60-250ml samples were filtered through Whatman GF/F filters, dried and analysed on a Hereaus (CHN rapid) Analyser, using cyclohexanone (10.14%N, 51.59%C) as a standard.

3.2.2.3 Fluorimetry

A relative measure of phytoplankton abundance was obtained from cell autofluorescence measured under ultra-violet light in a Turner fluorimeter. This appears here by permission of Ms. S.J. Painting.

3.2.2.4 Bacterial numbers and biomass

5ml aliquots were preserved with Analar glutaraldehyde (2.0% final concentration v/v) for counting of total cell numbers, according to the methods of Hobbie et al. (1977). Cells were counted using a Leitz Laborlux U.V. epifluorescent microscope, and were differentially assigned to one of 7 morphotypes, as described in Section 2.2.3.

3.2.3 Viable count and percentage plateability

5ml aliquots were taken from the mesocosm daily during the first 8 days of the incubation and then at 2-3 day intervals thereafter, for plating onto 0.1% Pep-SWA and 0.5% Pep-SWA (see Appendix). 100µl subsamples were plated (either at full strength or at an appropriate dilution to produce about 250 colonies per plate) in triplicate on each medium. Plates were incubated at 21°C for 10 days and stored at 4°C until counted. Plate counts included all colonies visible at 10x magnification under a Nikon 6CT2 profile projector. In addition, the number of chromogenic colonies (Cytophaga and Flavobacterium) was also counted.

3.2.4 Heterotrophic uptake of ^{14}C radiolabelled carbohydrates and amino acids

Rates of uptake of ^{14}C universally labelled glucose aspartic acid, glutamic acid and alanine by bacterial populations were estimated following the methods of

Wright and Hobbie (1965, 1966), Griffiths et al. (1977) and Wright (1978).

On days 2, 3, 5, 7, 9, 12, 15, 22, 25, 30, 38 and 43 of the incubation, 4 x 20ml aliquots of water were taken in sterile glass containers. These were prefiltered through Whatman GF/F filters to remove macro-plankton, and separately to each, a single ^{14}C radiolabelled substrate was added. The following substrates and final concentrations were used: glucose (11 $nmol.l^{-1}$), alanine (12 $nmol.l^{-1}$), aspartic acid (10 $nmol.l^{-1}$), and glutamic acid (10 $nmol.l^{-1}$). A 2ml subsample was taken immediately from each incubation, whereafter they were maintained in the dark at 12°C, and subsamples (2ml) were taken every hour for 6 hours. Subsamples were filtered through 0.2µm Gelman nitrocellulose filters and the filters were washed with a 0.1mmol solution of the appropriate substrate in sterile, filtered seawater to reduce non-specific adsorption of radiolabelled substrate to both cells and filter paper. Disintegrations per minute (D.P.M.) present on filters were measured by drying the filters, and covering them with 5ml of Instagel scintillation fluid in scintillation vials, which were stored in the dark for 24 hours before counting in a Packard Tricarb 460 liquid scintillation counter.

3.2.5 Plateable population characterisation

On days 1, 3, 5, 7, 16, 28, 34 and 42 of the incubation, 40 colonies were randomly picked from 0.1% Pep-SWA plates. These colonies were purified and characterised using the same tests and methods described in Section 2.

3.2.5.1 Data analysis

All data relating to characterisation of strains were analysed by multi-variate analysis to produce 1 and 2 dimensional displays of similarity, according to the Bray-Curtis measure of similarity, as described in Section 2. Taxonomic groups were defined at the 85%S level, and indices of diversity (Shannon-Weaver H', $D = {}^{N}/_{n} \times 100$) were calculated as described in Section 2. In addition, the Troussellier functional evenness index (E) was calculated, following the methods outlined in Troussellier and Legendre (1982).

3.3 Results

3.3.1 Relative fluorescence

Results of measurements of relative fluorescence are shown in fig.18. Initial relative fluorescence levels were low, showing that a low phytoplankton concentration

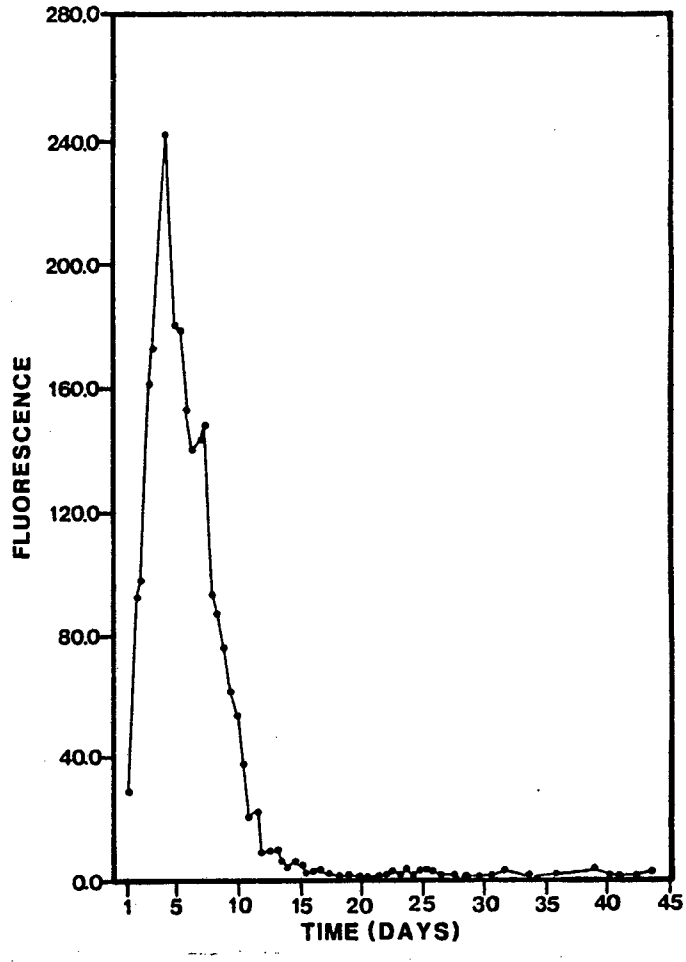

FIGURE 18: Changes in relative fluorescence during the 43 day incubation.

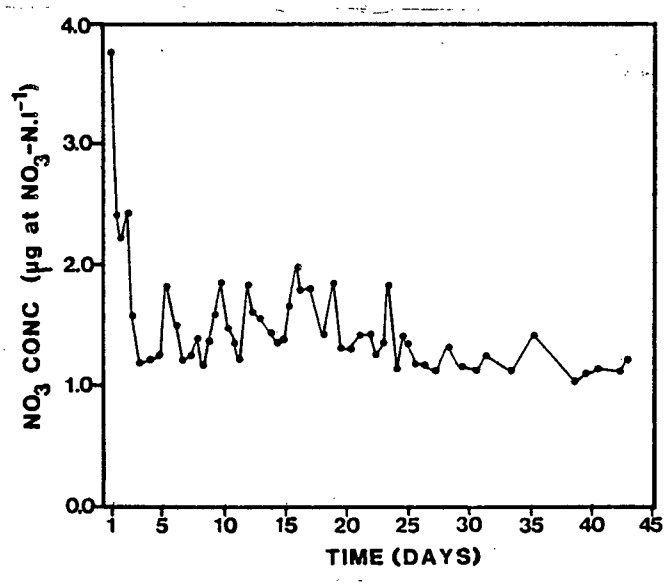

FIGURE 19: Changes in NO_3 concentration (μg at NO_3-$N.l^{-1}$) during the 43 day incubation.

was present, as is characteristic of newly upwelled water (Brown, 1981). The relative fluorescence rose rapidly to a peak of 245.0 on day 5. Thereafter the fluorescence decreased rapidly and by day 10 was only 10.0. It remained low for the duration of the experiment.

3.3.2 NO_3 concentration

Results of NO_3 measurement are shown in fig.19. Although the water temperature and relative fluorescence was low, suggestive of strong upwelling when water was collected, the initial NO_3 concentration was also low (4.0µg at NO_3-N.l^{-1}), suggesting that water was stage 2 upwelled water as described by Barlow (1984).

The NO_3 concentration fell rapidly over the first 3 days of the experiment, to 1.3µg at NO_3-N.l^{-1}. Thereafter the NO_3 concentration remained low for the duration of the experiment. From day 5 to day 23 periodic variations in the NO_3 concentration over approximately 5 day periods occurred, during which the concentration increased from 1.0µg at NO_3-N.l^{-1} to nearly 2.0µg at NO_3-N.l^{-1}. From day 23 until conclusion of the incubation, NO_3 concentrations remained close to 1.0µg.at NO_3-N.l^{-1}.

3.3.3 Dissolved Organic Carbon

Results of analysis of D.O.C. concentrations are presented in fig.20. It was difficult to obtain reliable estimates of D.O.C. concentrations, and wide fluctuations occurred. A certain amount of this variation may be ascribed to the diurnal fluctuation of phytoplankton activity (Carlucci et al., 1984; Fuhrman et al., 1985), but in addition it is suggested that bacterial uptake of D.O.C. may be very rapid, and that observed D.O.C. levels are the result of a complex interaction between phytoplankton productivity and heterotrophic uptake rates (Meyer-Reil et al., 1979; Burney et al., 1982; Carlucci et al., 1984; Fuhrman et al., 1985).

Initial D.O.C. levels rose to a maximum value (5.57µg at.l^{-1}) within 24 hours of the start of the inc- ubation. There after, D.O.C. levels fell rapidly and by day 6 were only 1.34µg at.l^{-1}. Again, as in the case of NO_3 levels, there appeared to be periodic variations in the levels of D.O.C.; between days 10 and 18, D.O.C. concentrations ranged between 1 and 3µg at.l^{-1}. Subsequent to this, they fell even further and ranged between 0 and 1.5µg at.l^{-1} for the duration of the enclosure.

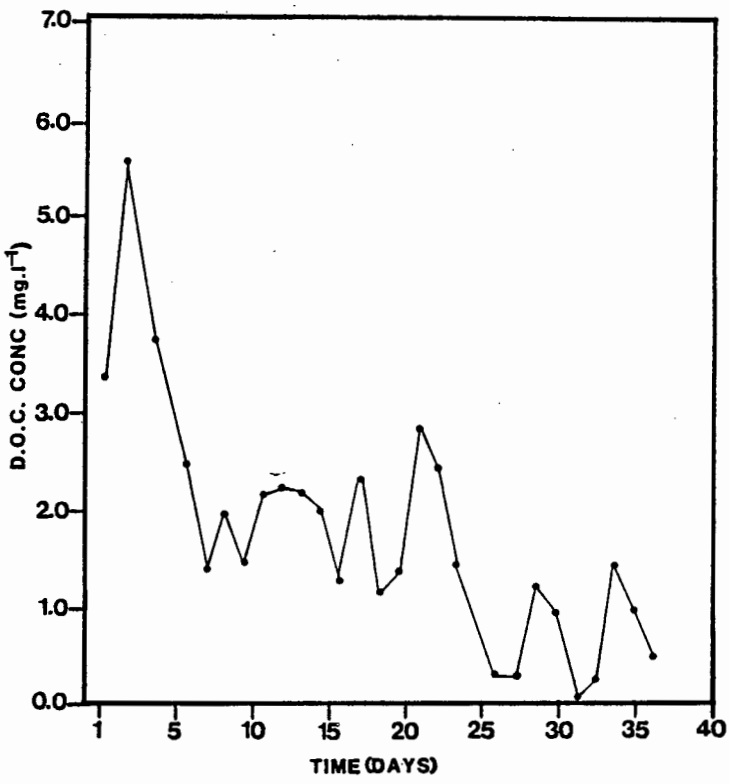

FIGURE 20: Changes in Dissolved Organic Carbon (D.O.C.) concentration ($\mu g\ at.l^{-1}$) during the 43 day incubation.

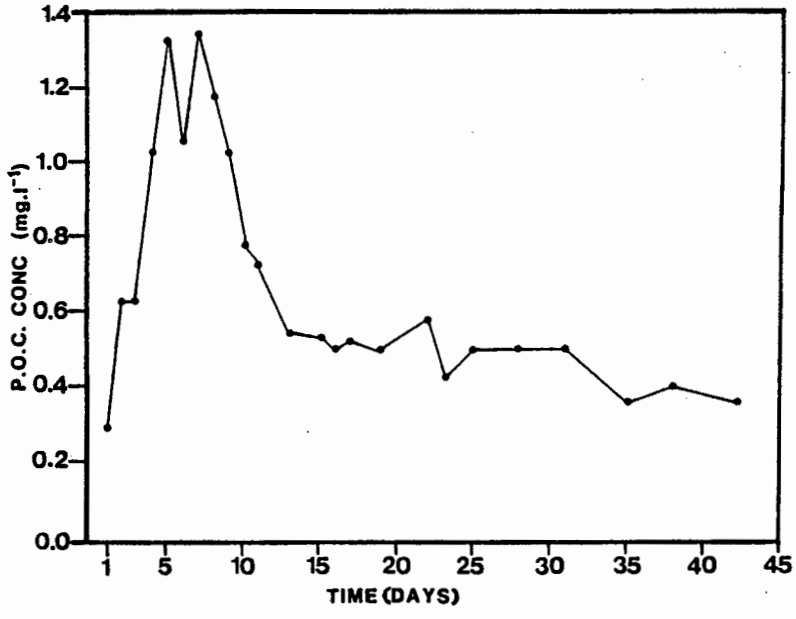

FIGURE 21: Changes in the Particulate Organic Carbon (P.O.C.) concentration ($mg \cdot l^{-1}$) during the 43 day incubation.

3.3.4. Particulate Organic Carbon

Results of analysis of Particulate Organic Carbon (P.O.C.) are presented in fig.21. The initial P.O.C. load was low (0.29 $mg.l^{-1}$), but there was an increase during the first 6 days of the incubation to a maximum of 1.35 $mg.\ell^{-1}$ on day 7. Thereafter there followed a period of rapid decline (0.17 $mg.\ell^{-1}.day^{-1}$) until day 13, whereafter a much slower decline in P.O.C. levels took place until the termination of the incubation (0.0042 $mg.\ell^{-1}.d^{-1}$), at which point P.O.C. levels were close to the original concentration (0.36 $mg.\ell^{-1}$).

3.3.5 Bacterial numbers - total count

Results of A.O.D.C. estimates of total numbers are presented in fig.22. Initial numbers were similar to those recorded previously (section 2) for upwelling water (2.9 x 10^6 $cells.m\ell^{-1}$). After a short (12h) lag, numbers increased approximately exponentially until day 3, to a maximum of 7.5 x 10^6 $cells.m\ell^{-1}$, remaining close to that level until day 5.

From day 5 to day 7, numbers fell precipitously to a minimum of 1.56 x 10^6 $cells.m\ell^{-1}$, remaining at that level until day 10. Thereafter, a slow increase in

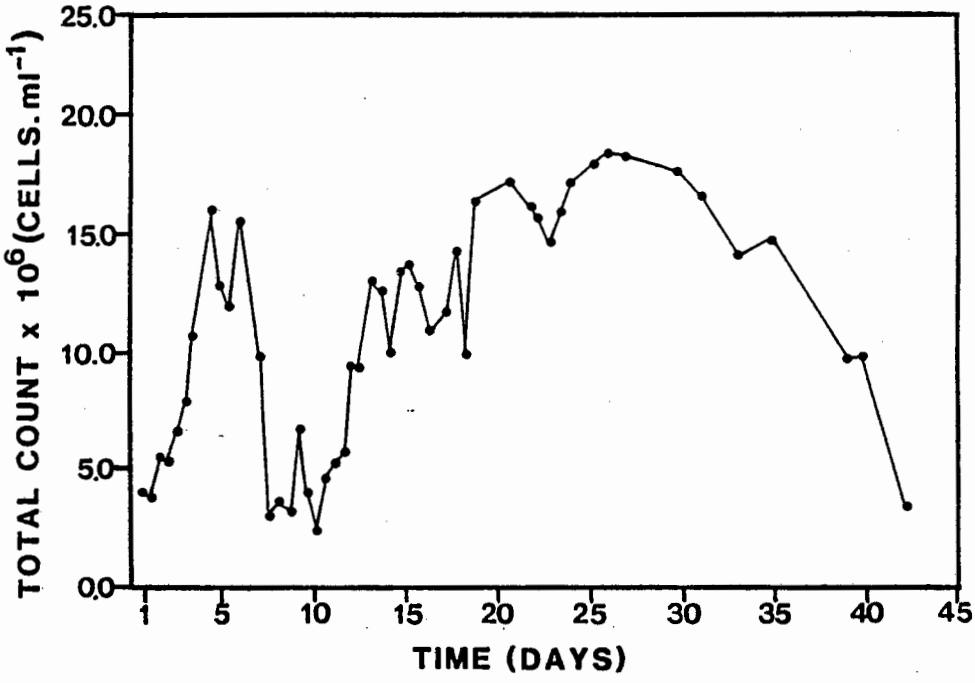

FIGURE 22: Changes in the total bacterial count (cells x $10^6.ml^{-1}$) during the 43 day incubation.

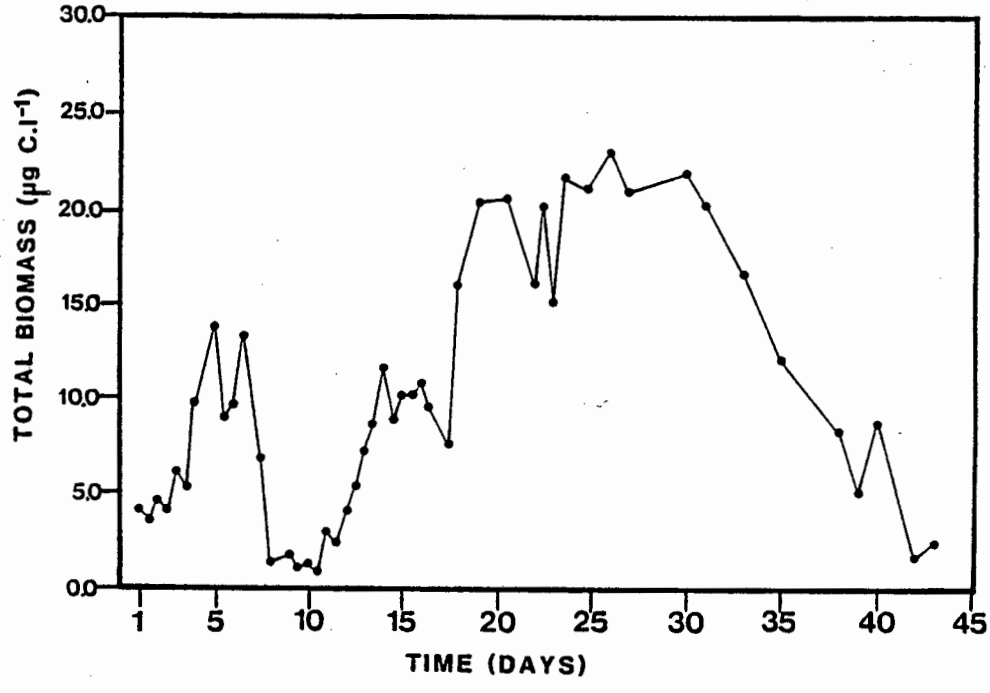

FIGURE 23: Changes in the total bacterial biomass (µg C.l^{-1}) during the 43 day incubation.

numbers occurred which persisted until day 27, peaking at 8.0 x 10^6 cells.$m\ell^{-1}$. From day 27 until day 43, a steady decline in numbers occurred and at the termination of the incubation, total numbers were close to the original recorded figure (1.8 x 10^6 cells. $m\ell^{-1}$).

3.3.6 Bacterial biomass - total biomass

Results of the estimation of total bacterial numbers from the differential counts and mean cell volumes of each morphotype (Section 2) are presented in fig.23. The total bacterial biomass throughout the incubation shows a similar bimodal distribution to that described for total numbers (fig.22). The biomass was initially low (40.08µg C.ℓ^{-1}) rising to a peak between days 3 and 5 of 136.05µg C.ℓ^{-1}. Between days 5 and 7 there was a rapid decline in total biomass to 17.00µg C.ℓ^{-1}, but this increased from day 10 to day 27 to a maximum of 240.00µg.ℓ^{-1}, after which it declined to 54.08µg C.l^{-1} at the termination of the experiment.

3.3.6 Bacterial numbers - differential counts

Results of differential counts for selected days of the incubation are presented as bar charts of the percentage

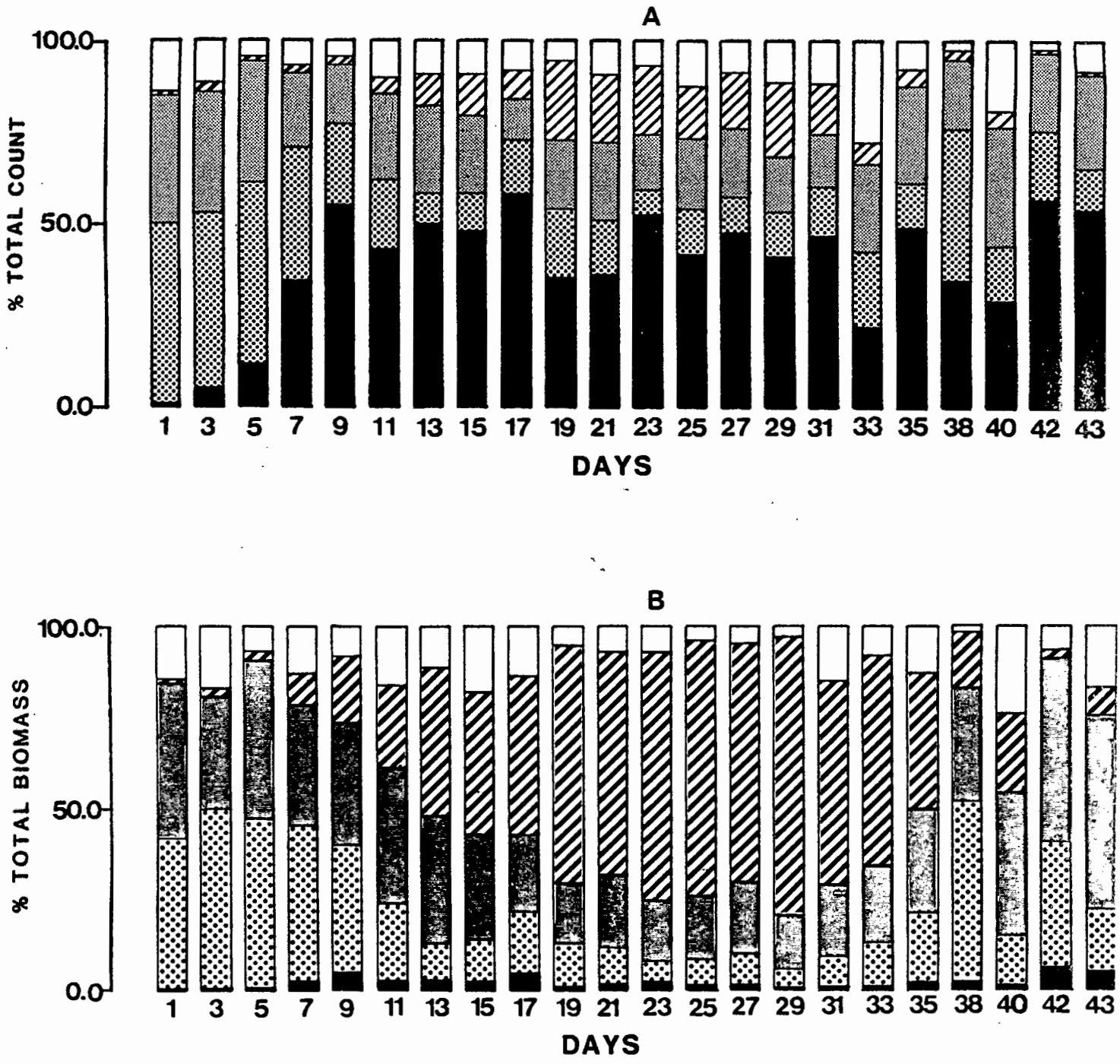

FIGURE 24: Changes in the proportion of the population comprised of small cocci (■), large cocci (⁘), small rods (▒), large rods (▨) and other morphotypes (□). (Fig. 24a: Proportion of total count. Fig. 24b: Proportion of total biomass).

composition of the total population by each morphotype (fig.24a). A striking feature was the relative rarity of small cocci (ultramicrobacteria) in the initial samples. These increased rapidly in relative importance, and by day 8 and for the duration of the incubation, they formed close to 50% of the population by number. It must be pointed out, however, that this may be a function of the efficiency of counting. True ultramicrobacteria (<0.3μm in diameter) are extremely difficult to count since they lie at the limit of resolution of the microscope, and their apparent increase in numbers may in fact reflect an increase in their size to levels which make them easier to count.

The initial population consisted primarily of large cocci (50.5%), small rods (37.5%) and other morphotypes such as spirillar and cyclical forms, with very few large rods being present. This situation was maintained for the first 4 days of the incubation, after which the increasing number of small cocci recorded reduced the relative proportion of other morphotypes, particularly during the drop in total numbers which occurred between day 5-10. The second population increase (days 10-27) was characterised by an increase in the relative numbers of large rods, which formed close to 20.0% of the population for much of the period of days 18-30, whereafter they declined to close to their original

level. Small rods, on the other hand, formed between 20-35% of the population through most of the incubation, while large cocci, though initially relatively abundant, ultimately formed a relatively small proportion of the total.

3.3.8 Bacterial biomass - differential estimates

Results of differential estimates of biomass of the various morphotypes are presented as bar charts in fig.24b.

Despite their numerical importance, small cocci contributed very little to the total recorded biomass throughout the incubation. Initially, the biomass distribution was dominated by large cocci (40.61%) and small rods (43.42%) with large rods making a very small contribution (1.83%) and the rest of the population (spirillar and cyclical forms) contributing 14.47%. This situation persisted for the first 6 days, and little change in distribution was noted through the initial population increase and small rods. With the decline in numbers between days 6 and 10, there was a decrease in the biomass of large cocci.

With the second population increase (fig.22) the relative importance of the biomass of large rods rapidly increased

and by day 28 they formed 64.71% of the total biomass, declining rapidly thereafter to close to the original level so that the final biomass distribution closely resembled that at the start of the incubation.

3.3.9 Colony forming units and percentage plateability

Changes in the viable count (as C.F.U.ml^{-1}), from plate counts on 0.1% and 0.5% Pep-SWA, are presented in figure 25. The %P, calculated from the viable count on 0.1% Pep-SWA is presented in fig.26.

On both media, the viable count was initially low (0.1% Pep-SWA = 1.33 x $10^3.m\ell^{-1}$, 0.5% Pep-SWA = 2.76 x $10^2.m\ell^{-1}$) but rose rapidly; the viable count on 0.1% Pep-SWA peaked on day 7 (3.67 x $10^5.m\ell^{-1}$) and that on 0.5% Pep-SWA on day 11 (2.78 x $10^5.m\ell^{-1}$), but declined thereafter to a minimum of 5.0 - 6.0 x $10^6.m\ell^{-1}$ on both media on day 15. Thereafter there was a slight increase in the viable count on both media, which peaked again on day 43 at 3.39 x $10^5.m\ell^{-1}$ on 0.1% Pep-SWA, and 2.52 x $10^5.m\ell^{-1}$ on 0.5% Pep-SWA. For the greater part of the incubation, the viable count on 0.1% Pep-SWA was higher than those on 0.5% Pep-SWA, the only exception to this occurring between days 9 and 15.

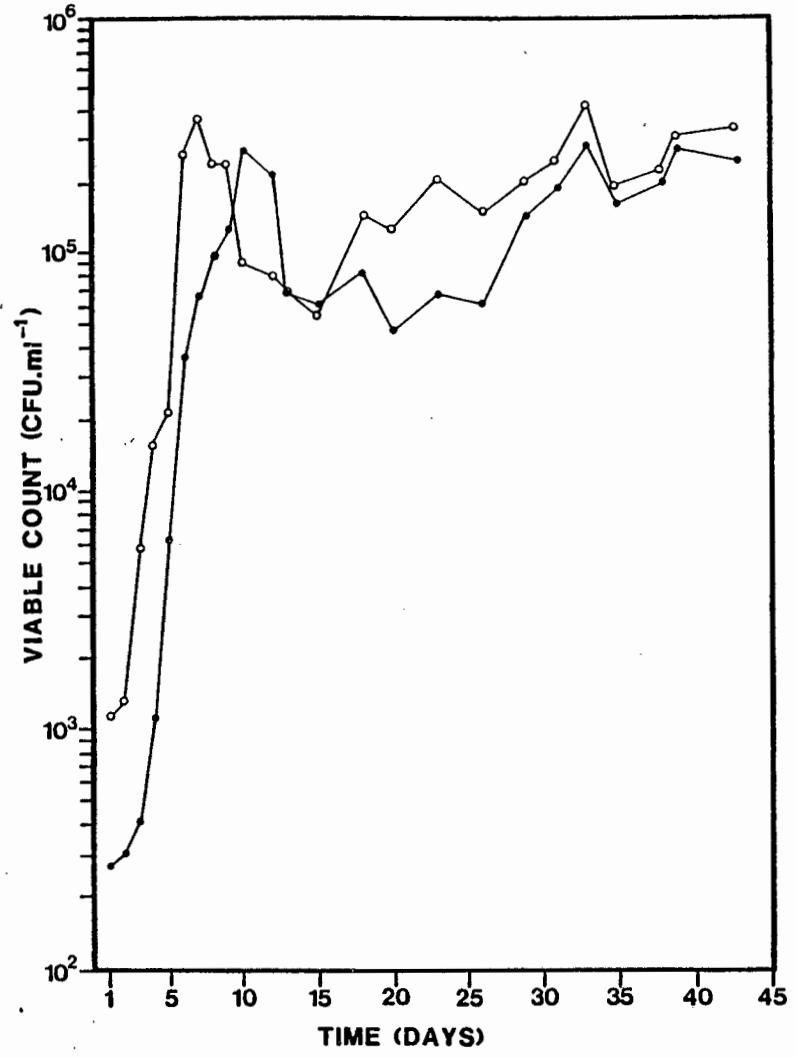

FIGURE 25: Changes in the viable count (CFU.ml^{-1}) assessed on 0.1% Pep-SWA (—o—) and 0.5% Pep-SWA (—●—).

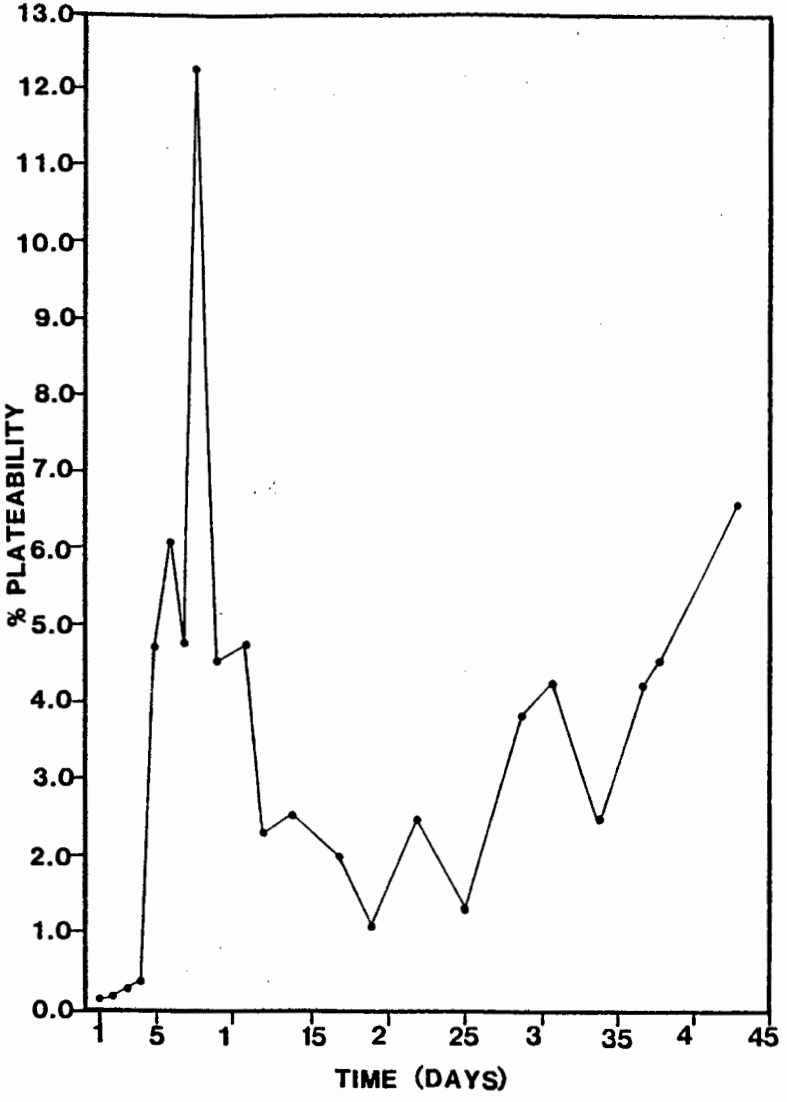

FIGURE 26: Changes in the percentage plateability assessed on 0.1% Pep-SWA

The %P was initially low (0.07%) and remained so for 4 days, increasing thereafter to a maximum of 12.30% on day 9. The %P then decreased rapidly to 2.30% on day 11, and continued to decrease more slowly to a minimum of 1.16% on day 19, whereafter it increased again (somewhat erratically) to 6.53% on day 43.

3.3.10 Heterotrophic uptake of ^{14}C radiolabelled substrates

Uptake rates were standardised for 1mg of bacterial carbon, using the biomass estimates obtained in Section 3.3.8, as recommended by Hoppe (1978), Wright (1978) and Simon (1985). Although uptake experiments were run for 6 hours, it was found that active uptake of ^{14}C radiolabelled substrates usually took place in the first 3 hours of incubation; thereafter, levels of uptake of ^{14}C radiolabelled substrate usually declined. Velocities of uptake were calculated from the linear uptake portions of these incubations. Specific velocities of uptake for glucose, glutamic acid, alanine and aspartic acid on each of the days tested are presented in figures 27a-d. Active uptake of all substrates was limited to the first 9 days of the incubation, and thereafter almost no active uptake could be demonstrated for any substrate.

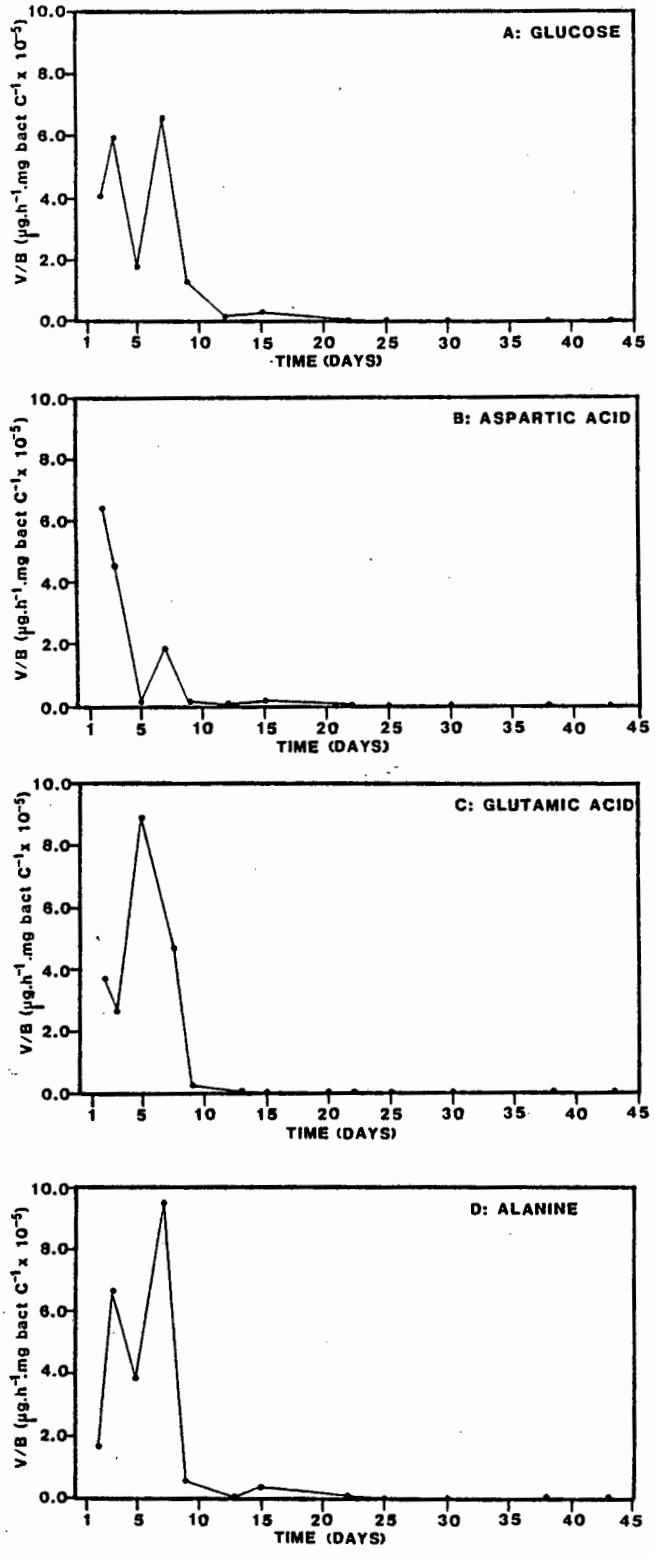

FIGURE 27: Changes in the specific uptake rates (V/B= $\mu g.h^{-1}.mg$ bacterial C^{-1}) during the 43 day incubation. (Fig. 27a: Glucose. Fig. 27b: Aspartic acid. Fig. 27c: Glutamic acid. Fig. 27d: Alanine).

3.3.10.1 Glucose

Active uptake of glucose was recorded on days 2, 3, 5, 7 and 9 of the incubation. The initial specific velocity of uptake (V/B) was high (4.09×10^{-4} µg.h^{-1}.mg C^{-1}) and increased to 5.96×10^{-4} µg.h^{-1}.mg C^{-1} by day 3. On day 5 V/B had decreased to 1.70×10^{-4} µg. h^{-1}.mg C^{-1} cells, but increased again to 6.60×10^{-4} µg.h^{-1}.mg C^{-1} in day 7. It decreased through day 9 (1.24×10^{-4} µg.h^{-1}.mg C^{-1}) to low levels of uptake until day 15 (2.98×10^{-5} µg.h^{-1} .mg C^{-1}) whereafter no further uptake was recorded.

3.3.10.2 Aspartic acid

The highest rates of uptake of aspartic acid occurred at the start of the incubation (day 2: V/B = 6.37×10^{-4} µg.h^{-1}.mg C^{-1} cells) decreasing through day 3 (V/B = 4.58×10^{-4} µg.h^{-1}.mg C^{-1}) to a minimum on day 5 (1.40×10^{-4} µ g.h^{-1}.mg C^{-1}). A slight increase occurred on day 7 (1.83×10^{-4} µg.h^{-1}.mg C^{-1}) but by day 9, uptake rates were again low (1.80×10^{-5} µg.h^{-1}.mg C^{-1}), and remained at this level until day 15, after which no further uptake could be detected.

3.3.10.3 Glutamic acid

Glutamic acid was initially taken up with a V/B = 3.64 x $10^{-4}\mu g.h^{-1}.mg\ C^{-1}$. A slight decrease to 2.61 x $10^{-4}\mu g.h^{-1}.mg\ C^{-1}$ occurred on day 3, but this increased again to 8.85 x $10^{-4}\mu g.h^{-1}.mg\ C^{-1}$ on day 5. Thereafter it decreased through day 7 (4.71 x $10^{-4}\mu g.h^{-1}.mg\ C^{-1}$) to very low levels on day 9 (2.63 x $10^{-5}\mu g.h^{-1}.mg\ C^{-1}$), after which no active uptake could be detected.

3.3.10.4 Alanine

Alanine was initally taken up with V/B = 1.61 x $10^{-4}\mu g.h^{-1}.mg\ C^{-1}$. On day 3, the rate of uptake increased to 6.69 x $.h^{-1}.mgC^{-1}\ 10^{-4}\mu g$, but decreased again by day 5 to - 3.88 x $10^{-4}\mu g.h^{-1}.mg\ C^{-1}$. On day 7 the rate of uptake was again high (9.50 x $10^{-4}\mu g.h^{-1}.mg\ C^{-1}$) but it decreased thereafter to 5.34 x $10^{-5}\mu g.h^{-1}.mg\ C^{-1}$ (day 9) and then to low levels of uptake on day 12 (4.39 x $10^{-6}\mu g.h^{-1}.mg\ C^{-1}$) and day 15 (3.22 x $10^{-5}\ \mu g.h^{-1}.mg\ C^{-1}$), after which no further uptake was recorded.

3.3.11 Population composition: discriminatory tests

A summary of the percentage of isolates showing a posi-

Table 6: Summary data matrix of percentage of mesocosm isolates showing a positive reaction to discriminatory tests.

Test	DAYS OF SAMPLING 1	3	5	7	16	28	34	43
Gram positive	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.3
Chromogenic	2.5	5.1	0.0	0.0	20.0	17.7	15.6	10.5
Coccoid	2.5	7.7	2.9	0.0	2.9	2.9	0.0	13.16
Motile	85.0	74.4	97.1	90.0	57.1	58.8	50.0	68.4
Caseinase +	35.0	25.6	88.2	33.3	20.0	38.2	0.0	0.0
Caseinase ++	20.0	0.0	29.4	23.3	2.9	0.0	0.0	0.0
Gelatin liq +	57.5	56.4	94.1	83.3	48.6	55.9	25.0	21.1
Gelatin liq ++	35.0	30.8	94.1	83.3	40.0	47.1	0.0	2.6
Agarase +	12.5	28.2	8.8	6.7	8.6	52.9	56.3	13.2
Agarase ++	0.0	18.0	8.8	6.7	8.6	11.8	11.8	0.0
C.M.C^{ase} +	0.0	5.1	14.7	10.0	0.0	0.0	3.1	0.0
C.M.C^{ase} ++	0.0	5.1	8.8	6.7	0.0	0.0	0.0	0.0
Oxidase	90.0	25.6	38.2	73.3	45.7	58.8	56.3	34.2
Catalase +	37.5	28.2	47.1	36.7	37.1	32.4	28.1	15.8
Catalase ++	12.5	20.5	38.2	20.0	25.7	20.6	17.6	0.0
Oxidative-glucose	40.0	33.3	14.7	36.7	22.9	5.9	12.5	23.7
Oxidative-mannitol	37.5	10.3	5.9	10.0	11.4	5.9	0.0	7.9
Fermentative-glucose	40.0	20.5	5.9	20.0	22.9	0.0	3.1	15.8
Fermentative-mannitol	32.5	15.4	2.9	0.0	2.9	0.0	0.0	13.2
NO_3 reductase +	57.5	30.8	20.6	23.3	17.1	23.5	18.8	13.2
NO_3 reductase ++	55.0	25.6	5.9	3.3	14.3	5.9	17.6	2.6
NO_2 reductase	2.5	0.0	0.0	0.0	0.0	0.0	3.1	0.0
Growth at 30°C +	100.0	76.9	97.1	83.3	68.6	97.1	87.5	86.8
Growth at 30°C ++	35.0	59.0	94.1	70.0	37.1	85.3	32.4	10.5
Growth at 37°C +	37.5	28.2	14.7	10.0	28.6	0.0	12.5	29.0
Growth at 37°C ++	17.5	5.1	5.9	3.3	14.3	0.0	2.9	5.3
Growth on Cetrimid-SWA	25.0	0.0	23.5	10.0	5.7	0.0	0.0	0.0
Growth on T.C.B.S.	32.5	7.7	2.9	0.0	0.0	0.0	0.0	7.9
n =	40	39	34	30	35	34	34	38

tive result to discriminatory tests on each of the days tested is presented in Table 6, and graphs of those showing a positive result to tests for motility, caseinase, gelatin liquefaction, agarase, C.M.C^{ase}, oxidase, catalase, NO_3-reductase, and acid production from glucose and mannitol are presented in figs 28a-l.

3.3.11.1 Gram strain

Gram positive isolates proved extremely rare throughout the incubation, and only one was recorded on day 43, apart from a cyanobacterial isolate recorded at the same time.

3.3.11.2 Motility

Throughout the incubation, the majority of isolates tested were motile. The population on day 1 had a high percentage of motile isolates (84.0%) but this fell slightly to 74% on day 3, rising to 97% on day 5, and thereafter decreased to between 50% and 60% until day 34, after which a slight increase to 68% was noted on day 43.

3.3.11.4 Caseinase production

Only 35% of isolates initially digested casein, but by

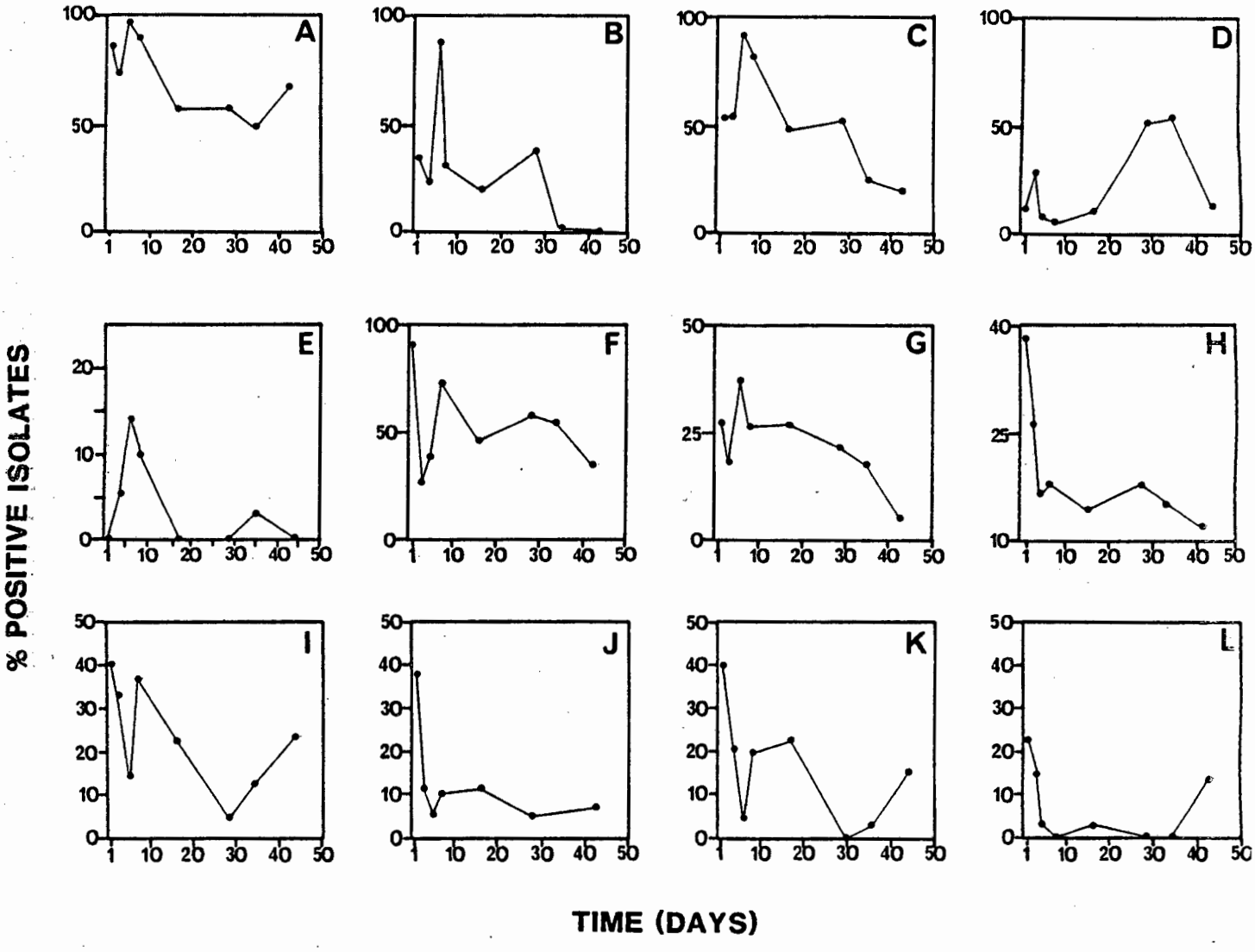

FIGURE 28: Changes in the proportion of isolates showing a positive reaction to 12 discriminatory tests. (Fig. 28a: motility. Fig. 28b: Caseinase production. Fig. 28c: Gelatin liquefaction. Fig. 28d: Agarase production. Fig. 28e: Carboxymethylcellulase production. Fig. 28f: Oxidase production. Fig. 29g: Catalase production. Fig. 28h: Nitrate reduction. Fig. 29i: Oxidative acid production - glucose. Fig. 29j: Oxidative acid production - mannitol. Fig. 29k: Fermentative acid production - glucose. Fig. 29l: Fermentative acid production - mannitol.)

day 5, 88% of isolates produced zones of clearance on milk agar plates. Thereafter a rapid decrease to close to the initial levels occurred and persisted till day 28, whereafter no further caseinase producing isolates occurred.

3.3.11.5 Gelatin liquefaction

A similar pattern of distribution of gelatin liquefaction was noted to that of caseinase production, although it was generally rather more common throughout. 57% of the initial population liquefied gelatin. By day 5 this had risen to 94%, and this high level persisted to day 7 whereafter progressively fewer isolates showed gelatin liquefaction, until, by day 42, only 21% of the isolated population gave a positive reaction.

3.3.11.6 Agarase production

Initially, a low percentage (12.5%) of isolates showed positive agarase activity. This rose to 28% on day 3, but fell to very low levels (<10%) on days 5, 7 and 16. Thereafter it increased and on days 28 and 34 the populations were dominated by agarase producing isolates (>50%).

By day 43, the frequency of agarase production had again decreased to close to the original level (13%).

3.3.11.7 Carboxymethylcellulase (CMC^{ase}) production

Carboxymethylcellulase production was fairly rare throughout the incubation. No CMC^{ase} producers were found in the initial population, but by day 3, 5% of isolates were found to be positive. The frequency increased through day 5 (14%) to day 7 (10%), but thereafter only rarely occurred, with only one isolate on day 34 showing positive activity.

3.3.11.8 Oxidase

Oxidase positive isolates dominated the initial assemblage (90%) but this situation was reversed rapidly by day 3, when only 25% of isolates were positive. Thereafter, the percentage of oxidase positive isolates increased till they again dominated the assemblage on day 7 (73%). Their occurrence fell by day 16 (45.71%) but they were again in the majority on days 28 (58.82) and 34 (56.25), but rather fewer were present on day 43 (34.21).

3.3.11.9 Catalase

Throughout the experiment, catalase negative isolates dominated the assemblages. Initially, 37.50% of isolates

were catalase positive - this rose to 47% of isolates on day 5, but thereafter progressively decreased, till by day 43, only 15.8% of isolates were positive.

3.3.11.10 Oxidative acid production from carbohydrates

3.3.11.10.1 Glucose

Initially, 40% of isolated isolates produced acid from glucose under aerobic conditions. This fell to 14.7% by day 5, but on day 7, an increase to 36.7% was noted. This decreased to only 5.88% of isolates on day 28, but thereafter increased again to 23.7% of strains on day 43.

3.3.11.10.2 Mannitol

On day 1, a similar percentage of strains produced acid from mannitol (37.5%) and glucose (40%) but by day 3 this fell to 10.3% and thereafter to 5.9% on day 7. A slight increase in frequency was noted on day 7 (10%) and 16 (11.4%) but fell again on day 28 (5.9%) and day 34 (0%). Mannitol oxidisers reappeared on day 43, comprising 7.9% of the total population.

3.3.11.11 Fermentative acid production carbohydrates

3.3.11.11.1 Glucose

A similar trend to that of oxidative production of acid was noted. Initially, 40% of strains produced acid from glucose under anaerobic conditions. This fell to 20.5% on day 3 and only 5.8% on day 5. An increase was noted to 20% on day 7 and 22.9% on day 16, whereafter fermentative strains were not present in high numbers on day 28 (0%) or 34 (3.13%) but reappeared in the assemblage only on day 43 (15.8%).

3.3.11.11.2 Mannitol

A similar trend to that of oxidative isolates was noted for mannitol fermentative isolates. Initially, 32.5% of the population fermented mannitol. This fell to 15.4% on day and continued to decrease through day 5 (2.9%) and 7 (0%) but appeared at a low level on day 16 (2.9%) and thereafter remained absent until day 43 (13.2%).

3.3.11.12 Nitrate reductase

Initially, the majority of strains reduced NO_3 (57.5%) but this fell rapidly through day 3 (30.8%), 5

(20.6%) and 7 (23.3%) to reach very low levels (13.2%) by day 43.

3.3.11.13 Nitrite reductase

NO_2^- reductase activity was very uncommon throughout the incubation, appearing only initially (2.5%) and on day 34 (3.13%).

3.3.12 Generic distribution

3.3.12.1 Cytophaga/Flavobacterium plate counts

The mean number of colonies on each day of the experiment which could be assigned to the Cytophaga/Flavobacterium complex on the basis of their chromogenic character are shown in fig.29, expressed as a percentage of the total number of C.F.U.

There is a bimodal distribution - Cytophaga/Flavobacterium initially comprised 2.5% of the isolates, rising to 8.3% on day 4, then falling to low levels for the succeeding 4 days. From day 8 - 18 there was a rapid increase in the number of colonies and they came to comprise 24.6% of all colonies on day 18, falling thereafter to 9.26% on day 43.

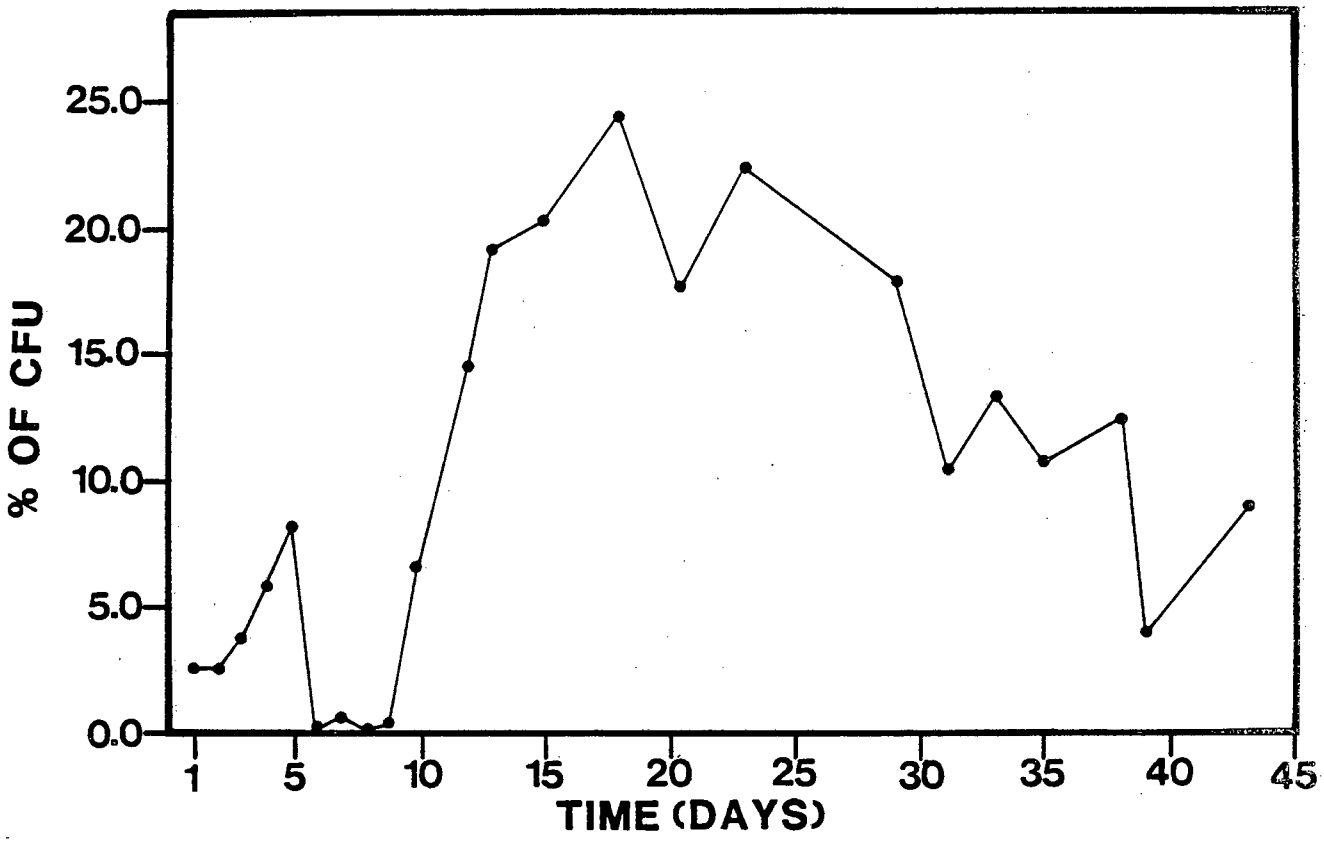

FIGURE 29: Changes in the proportion of chromogenic isolates (Cytophaga/Flavobacterium) assessed on 0.1% Pep-SWA during the 43 day incubation.

3.3.12.2 Generic distribution determined by random sampling

The complete generic distributions, for each of the days sampled, are presented in fig.30.

The initial sample was dominated by fermentative forms (Vibrio = 48.1%, Enterobacteriaceae = 23.9%) with non fermentative forms (Pseudomonas, Acinetobacter, Cytophaga/Flavobacterium and unidentifiable strains) comprising only 28.0% of the population.

This distribution changed very rapidly, and by day 3, the obligate aerobic strains dominated the assemblage. This situation was maintained for the duration of the incubation.

On day 5 only 2 genera were represented - Pseudomonas (93.5%) and Vibrio (6.5%). This correspended with the peak in numbers and biomass of bacterial cells shown in figs 22 and 23.

From day 5 to day 7 there was a drop in the total number and biomass of cells, and there was a corresponding change in the generic distribution - the percentage of facultative anerobes increased (22.7%) and a few Acinetobacter strains appeared (3.5%), but the community

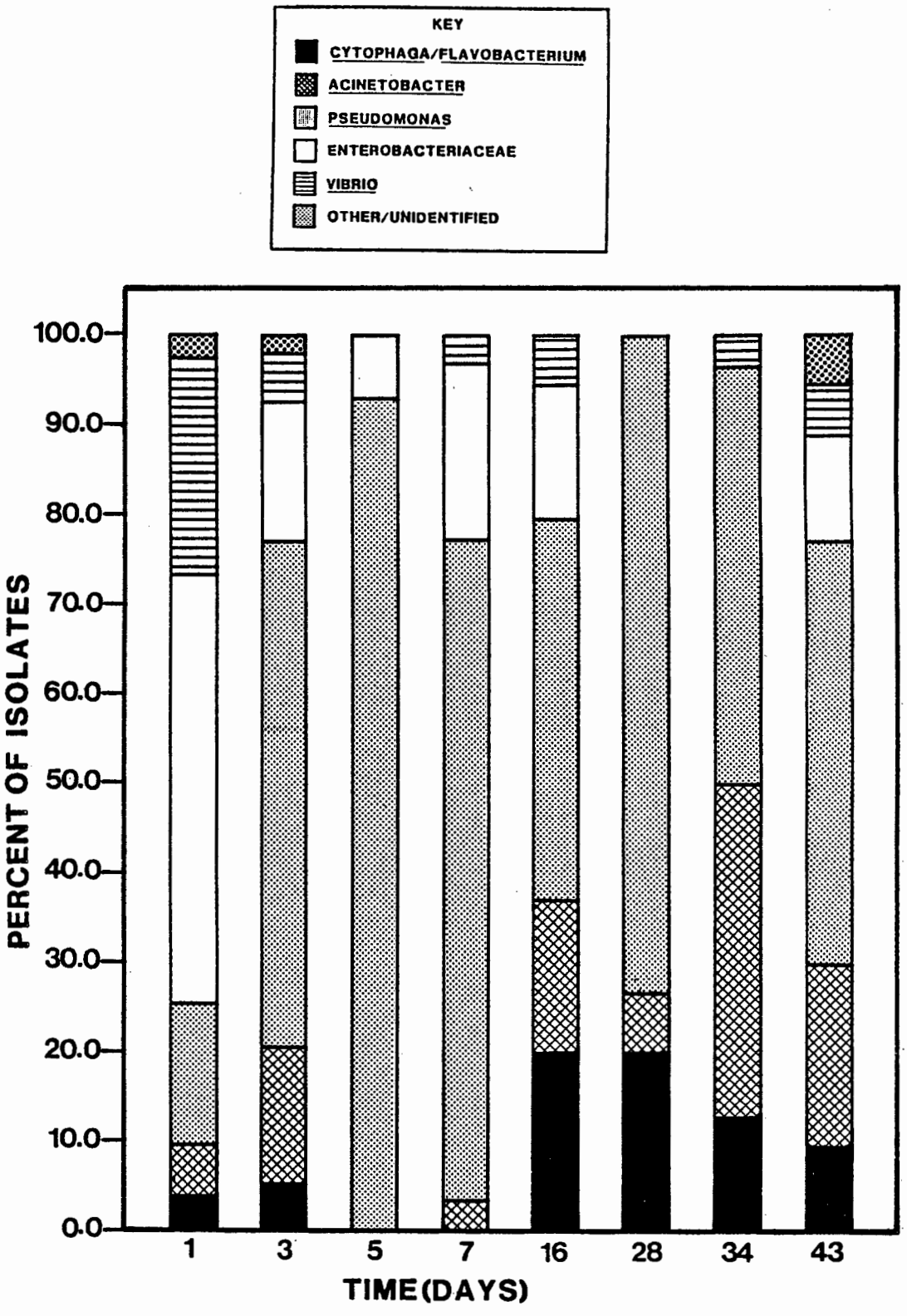

FIGURE 30: Changes in the generic composition of the plateable population during the 43 day incubation.

was dominated by Pseudomonas (74.2%).

Days 10 to 25 marked an almost exponential increase in numbers and biomass of bacterial cells: by day 16, the number of Cytophaga/Flavobacterium cells had increased markedly, as had the number of Acinetobacter strains, with facultative anaerobic isolates comprising only 20% of the total population and by day 28, these had again disappeared from the population. At this point, which appeared to be a stationary phase in the numbers and biomass increase, Pseudomonas again dominated the assemblage (73.3%) with Vibrio and Enterobacteriaceae reappearing only during the decline in population from day 28 on. This period (day 28 - 43) was further characterised by the increasing importance of the Acinetobacter isolates.

By day 43 the community structure closely resembled that of day 3 of the incubation: both groups of facultative anaerobes were present, as well as a small number of Cytophaga/Flavobacterium strains, with the assemblage being dominated by Pseudomonas (47.1%) and Acinetobacter (20.5%); interestingly there was also a single Cyanobacterial isolate, as well as one gram positive coccus.

The frequency of occurrence of Cytophaga/Flavobacteria strains in the randomly picked collections closely paralleled their frequency assessed by plate counts. This

suggests that the random selection method provides a good representation of strains present on solid media.

3.3.13 Multivariate analysis

3.3.13.1 Individual days

As an example of classification dendrograms and multi-dimensional scaling ordination plots used in diversity analysis, results for all strains tested on day 1 of the incubation are presented in figs 31a,b. In general, most strains tested were similar at the 40<-<50% similarity level. Thereafter, groups devolved at varying similarity levels - it was felt that, in common with populations discussed in Section 2, this was a function of the diversity within the populations. It was noted that the previous generic groupings used (Section 3.3.12) correlated roughly with the group presentations at around 60<-<70%S, but a higher level of discrimination (85%S) was used to determine physiological taxa within the population. It proved difficult to assign particular characteristics to each of these groups, since they are the product of an averageing process of discrimination, but as an example, the broad definitions of groups for day 1 are presented (Table 7). The number of groups present at the 85%S level are shown in Table 8.

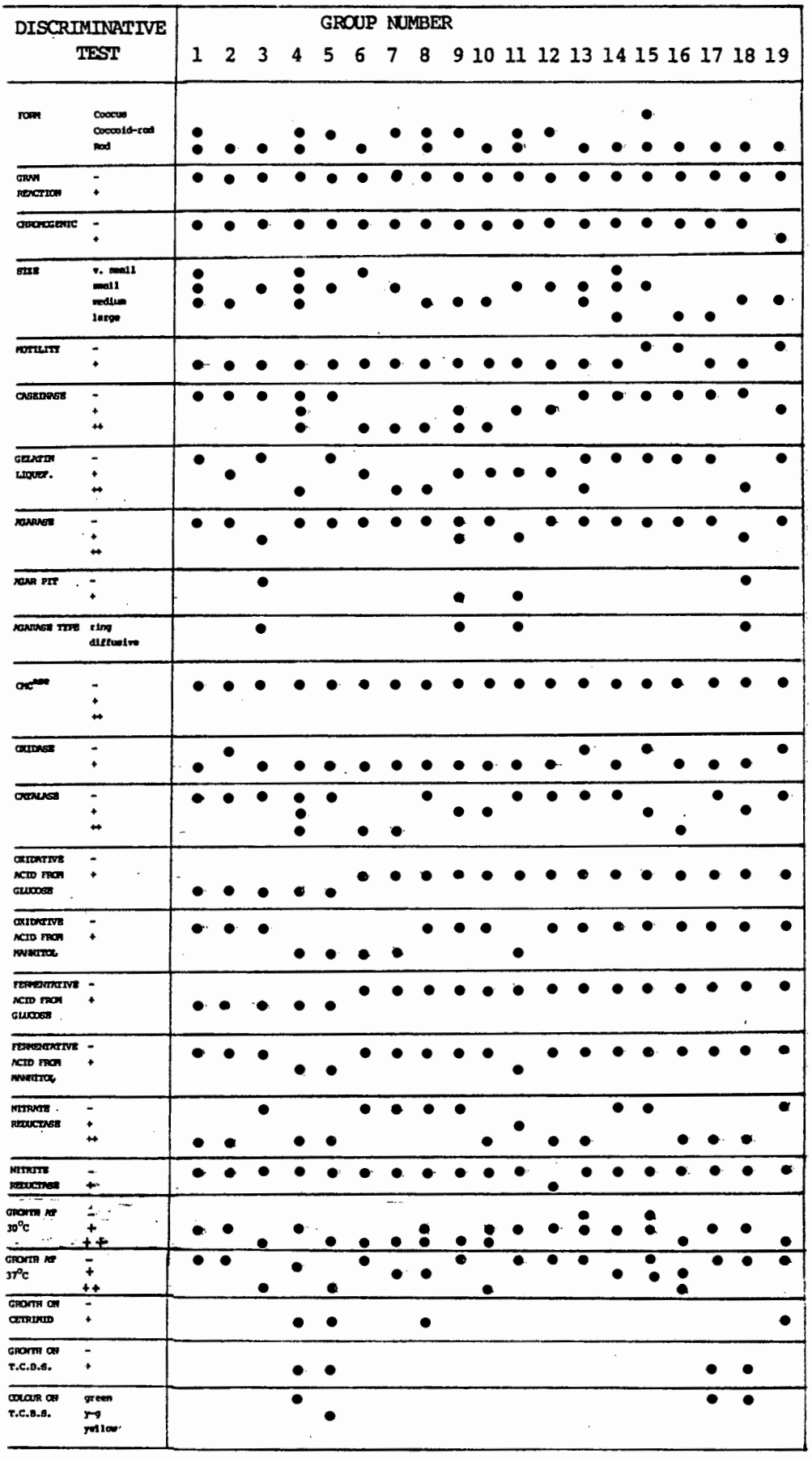

Table 7: Characteristics of groups present on day 1 of the mesocosm enclosure, defined at the 85% S level by classification according to the Bray-curtis measure of similarity.(● Denotes at least one member of the group showing a positive reaction to the test indicated.)

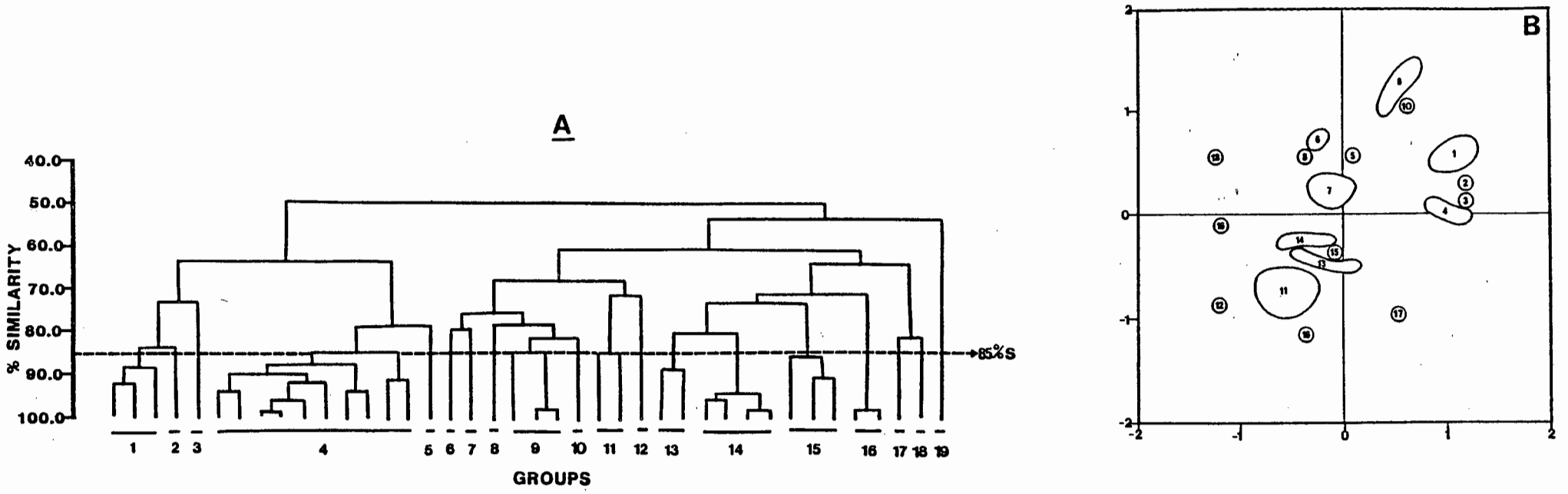

FIGURE 31: Results of similarity analysis of the randomly selected plateable population on Day 1 of the 43 day incubation. (Fig. 31a: classification dendrogram Fig. 31b: Multi-dimensional scaling ordination plot).

	DAYS OF SAMPLING							
	1	3	5	7	16	28	34	43
Number of isolates(n)	40	39	34	30	35	36	32	38
Number of groups at 85%S (N)	19	24	10	10	18	13	12	17

Table 8: Number of groups present at 85%S level for 8 days of the 43 day incubation

It was observed that three areas of high group presentation occurred: these correspond to days 1 - 3, day 16 and day 43, with low group presentations on both days 5 and 7.

3.3.13.2 Summary classifications

Results of classification analysis of the summary data matrix for days 1, 3, 5, 7, 16, 28, 34 and 43 of the incubation (Table 6) are presented in fig.32.

Day 1 of the incubation remained isolated and unique in the similarity analysis, its closest similarity occurring at the 66.5%S level with the populations of days 3, 16 and 43. A close grouping between days 3 and 16 occurred

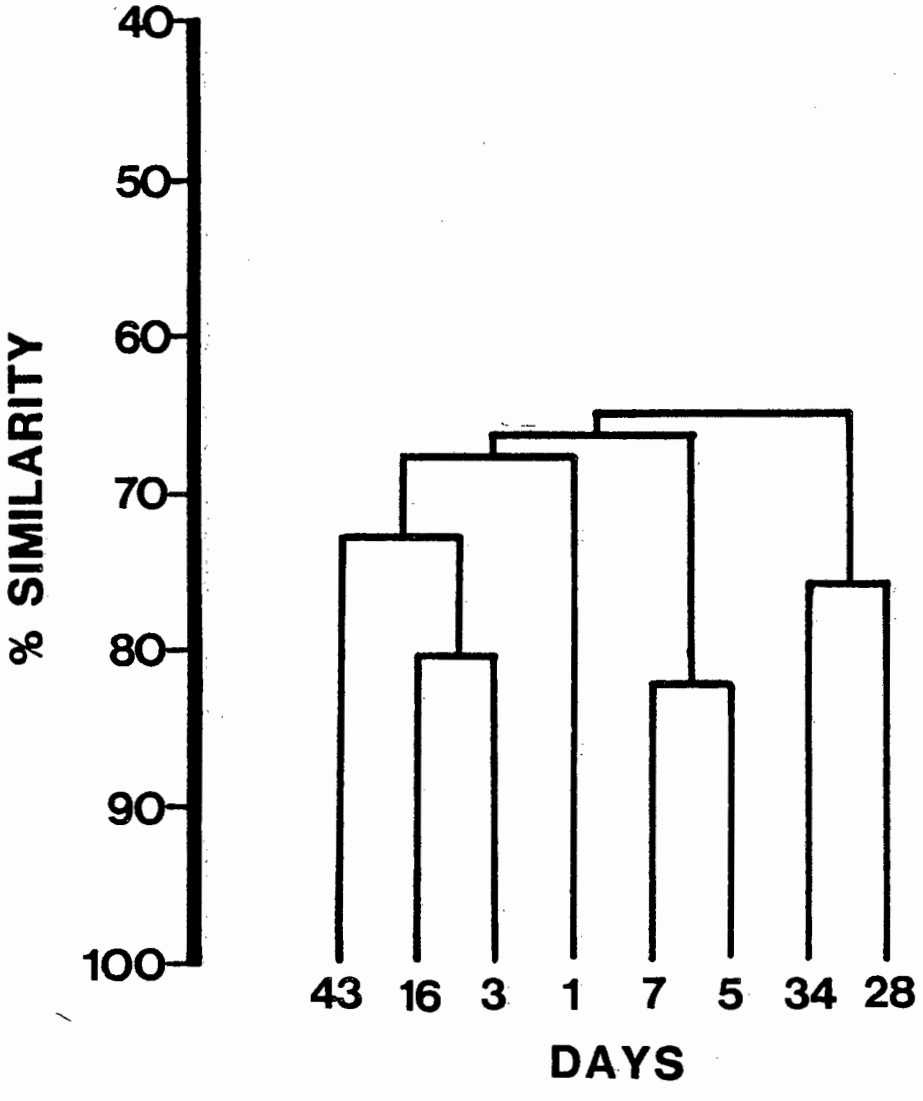

FIGURE 32: Results of similarity analysis of properties of the bacterial populations on 8 days of the 43 day incubation, based on the summary data matrix (Table 6).

at 82%S - both of these populations were derived from early log phase growth (fig.22). The population of day 43 was similar to these populations at the 73%S level.

The populations of days 5 and 7 proved very similar to one another at 85%S, and those of days 28 and 34 were paired at 76%S. Although both of these pairings were from similar stages of population development (late log - stationary phase) they remained quite dissimilar from one another.

3.3.14 Population diversity

Results of diversity analyses are presented in figs 33a-c. The initial diversity measures were high, and a slight increase was noted between days 1 and 3 in both H' and D: thereafter a decrease in these indices of diversity occurred, which was maintained between days 5 and 7. With the start of the second population increase, an increase in diversity to the same level as the initial values occurred, but this declined during the population peak, and was maintained for the duration of the incubation. The Shannon-Weaver index (H') showed very similar trends to that of D, except at the termination of the enclosure, where D increased while H' remained low. The value of E decreased slightly detween days 1 and 3, and then rapidly between days 3 and 15; as was the case for both H' and D,

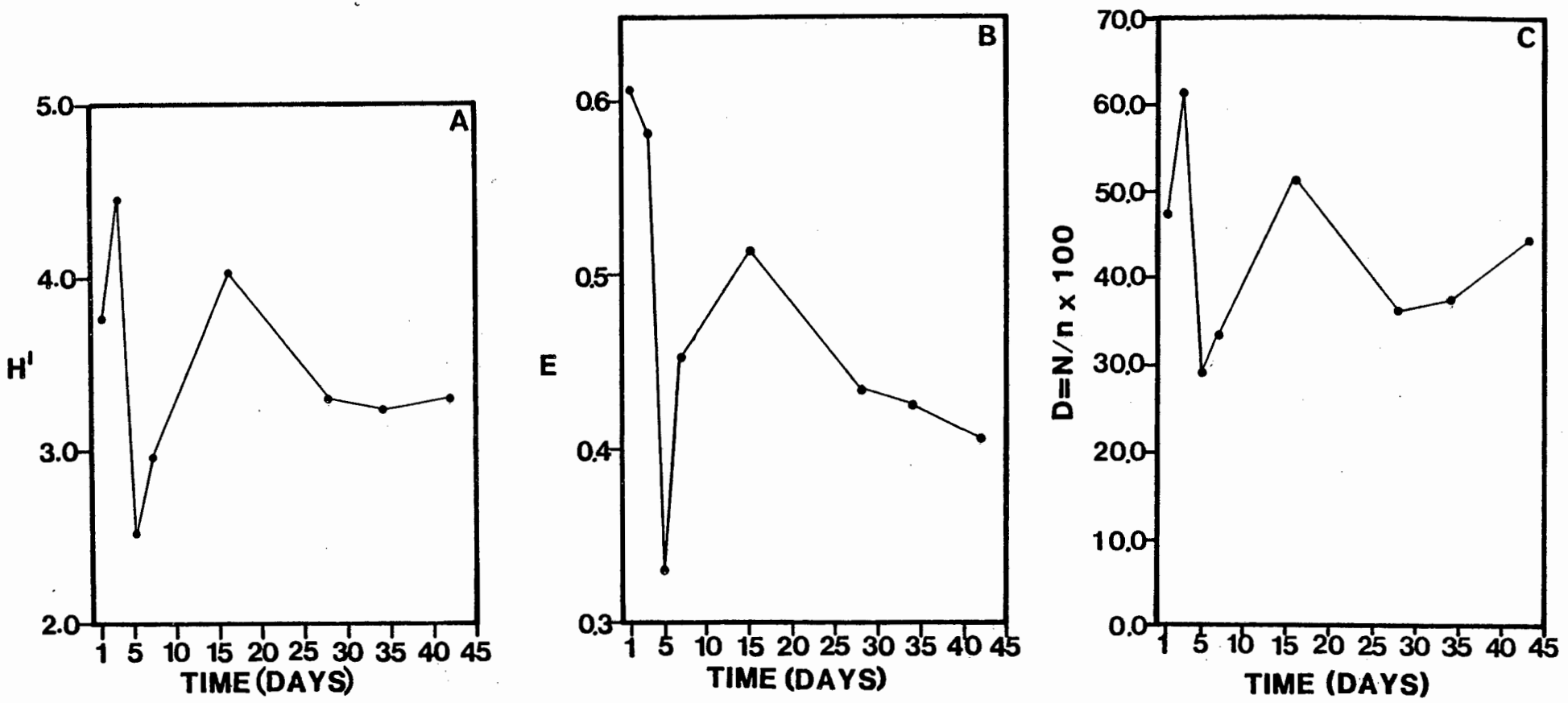

FIGURE 33: Changes in population diversity during the 43 day incubation. (Fig. 33a: Shannon-Weaver index (H'). Fig. 33b: Trousselier-Legendre functional evenness index (E). Fig. 33c: D = N/n x 100).

an increase in the value of E occurred between days 5 and 16, decreasing again between days 16 and 43.

3.4 DISCUSSION

The 43 day incubation was divided into two distinct periods, each denoted by the development and decline of a characteristic bacterial population. The first period lasted from day 1 to day 10 of the incubation, and the second from day 11 to the termination of the experiment.

Between days 1 and 6, a phytoplankton bloom developed in the mesocosm as a result of the exposure of nutrient rich upwelled water to light. Bloom development was rapid, as has previously been reported by Barlow (1982), and occurred at the expense of inorganic nutrients, in particular NO_3 (fig.19) whose decline closely followed the increase in fluorescence.

As the phytoplankton bloom developed, the D.O.C. concentration was observed to increase (fig.20), in keeping with reports that between 5% and 50% of photosynthetically fixed carbon may be released as dissolved exudates (Thomas, 1971; Ignatiades and Fogg, 1973; Itturiaga and Hoppe, 1977; Lancelot, 1979; Larsson and Hagstrom, 1979, 1982). This release occurred principally during exponential growth of the phytoplankton. Difficulties

pertain to the measurement of D.O.C. however, since it is rapidly taken up by bacteria, which distorts the observed concentration (Billen et al., 1980; Hagstrom et al., 1984). There is some controversy surrounding the mechanisms governing exudate release. Eberlein et al., (1983) noted that D.O.C. was principally released at the end of exponential growth phases, but Itturiaga and Hoppe (1977), Larsson and Hagstrom (1982) and Lancelot (1983) suggest that exudation in healthy phytoplankton cells is low (<10%) of photosynthetically fixed carbon, and Fogg (1966, 1971) Ignatiades and Fogg (1973) and Eberlein et al. (1983) suggest that extracellular release is greatest during periods of nutrient and light limitation, when carbon fixed during continuing photosynthesis is released as exudates instead of being incorporated into biomass (Fogg, 1966; Anderson and Zeutschel, 1970).

D.O.C. is rapidly and efficiently utilised by bacteria (Billen et al., 1980; Bolter, 1981; Cole et al., 1982; Laake et al., 1983b; Hagstrom et al., 1984; Ducklow et al., 1986), consisting of a variety of compounds, of which glucose and other carbohydrates (Hellebust, 1970; Eberlein et al. (1983) and dissolved free amino acids (Crawford et al., 1974; Brockmann et al., 1979) are the most important constituents. Both glucose (Wright and Hobbie, 1965, 1966; Griffiths et al., 1977) and amino acids (Crawford et al., 1974; Bright and Fletcher,

1983a, b; Eberlein et al., 1983) are taken up and incorporated or utilised efficiently by marine bacteria, and close correlations have been drawn between levels of D.F.A.A. and bacterial productivity (Bada and Lee, 1977; Palumbo et al., 1983; Laanbroek et al., 1985) as well as between total D.O.C. release and bacterial productivity (Bolter, 1981). Rates of uptake of radiolabelled glucose and amino acids are commonly used as indicators of heterotrophic bacterial activity (Wright and Hobbie, 1965, 1966; Vaccaro and Jannasch, 1966; Takahashi and Ichimura, 1971; Andrews and Williams, 1971; Crawford et al., 1974; Hoppe, 1977; Bright and Fletcher, 1983a, b; Simon, 1985).

In this instance, specific velocities of uptake of alanine, glutamic acid and glucose were high during this period, and increased during the development of the phytoplankton bloom, peaking at the time of highest phytoplankton abundance. It was noted, however, that the uptake of aspartate, which was high initially, decreased during this time. High specific velocities of uptake of alanine, glutamic acid and glucose were maintained until the decline of the phytoplankton bloom (figs 27a-d). As noted by Brockmann et al. (1977, 1979) the development of phytoplankton blooms is accompanied by changes in taxonomic structure which reflect in changes in the production of amino acids, which may occasion different preferential rates of heterotrophic uptake.

Billen et al. (1980) however, suggest that bacterial uptake is the principal determinant of observed D.F.A.A. concentrations, and in the absence of determinations of natural glucose and D.F.A.A. concentrations, both rates of uptake and specific velocities of uptake are likely to be underestimated (Crawford et al., 1974); nonetheless, there was evidence during this period of some sequential development of substrate preferences, and heterotrophic uptake was, in general, high.

Accompanying the rapid uptake of dissolved organic substances, a marked increase in bacterial numbers occurred until day 6 (fig.24a) mainly amongst the larger cocci and small rods. There was, however, little change in the overall biomass distribution (fig.24b) although the total biomass increased to a maximum between days 4 and 6 (fig.23). Such increases, which correlate with phytoplankton bloom development, have previously been reported (Simon, 1985; Laanbroek et al., 1985) and confirm the finding of Bird and Kalff (1984) that good correlations can be drawn between bacterial abundance and Chl a concentrations. An interesting feature during the development of the phytoplankton bloom was the increase in numbers of very small cocci. This appears to be paradoxical, since the production of small coccoid forms is generally believed to be symptomatic of the onset of

highly oligotrophic conditions (Torrella and Morita, 1982; Amy et al., 1983) South Atlantic Central Water has been shown to have a bottom time 500 years (Morita, 1984b; Shannon, 1985b) and hence newly upwelled water contains very low concentrations of D.O.C. Bacterial populations in newly upwelled water have therefore been subjected to long periods of starvation and small coccoid forms are commonly found in such water. Very small coccoid cells are, however, difficult to count by conventional A.O.D.C. techniques, since they lie at the limit of the resolving power of the microscope. Amy et al. (1983) and MacDonell and Hood (1982) have shown that such cells can be revived, and an increase in cell size is noted under conditions of gradually increasing ambient nutrients. As levels of D.O.C. rose during phytoplankton development, these small dormant forms were revived, and increased sufficiently in size to be accurately counted - the increase in numbers of such small cells is therefore believed to be artefactual.

Accompanying the increase in bacterial numbers and biomass was an increase in both the viable count and the %P, which, as suggested by Laake et al. (1983b), was felt to reflect the increasing nutritional competence of the population as nutrient levels increased in the enclosure. A lag of 2 - 3 days between increases in the cell count and increases in the C.F.U. and %P was

evident (figs 25, 26).

With the rapid development of the phytoplankton bloom, NO_3 levels fell to limiting levels of close to 1µg $at.l^{-1}$ (Barlow, 1984) and there was a decline in phytoplankton abundance between days 7 and 10. Accompanying the phytoplankton population decline was a rapid drop in bacterial numbers and biomass, which occurred principally among the large cocci and small rods, but with an increase occurring amongst the small cocci. Linley and Newell (1981) noted a similar decrease in numbers and biomass of bacteria following blooms associated with the degradation of kelp mucilage and phytoplankton debris, and showed that this occurred as a result of grazing by micro- flagellates. Such predation has been widely reported and micro-flagellates are generally believed to regulate bacterial population size, as well as enhancing metabolic activity in surviving cells by excretion of organic and inorganic nutrients and promoting D.O.C. release during phagotrophy (Fenchel, 1968, 1980a, b; Laake et al., 1983a, b; Mallory et al., 1983; Davis and Sieburth, 1984; Gray et al., 1984; Gast, 1985; Taylor et al., 1985; Ducklow et al., 1986). The increase in real and relative abundance of small cocci suggests that the smaller bacteria enjoy a selective advantage during predation, but in addition, this increase may be due to

a fragmentation response by the larger size classes as a result of the onset of low nutrient conditions, as has been described by Novitsky and Morita (1976, 1977, 1978), Tabor (1981), Torrella and Morita (1982) and Amy et al. (1983). In addition to these changes, there were declines in both the viable count and percentage plateability and in all estimates of heterotrophic uptake activity, which suggests a generalised starvation-survival response in the population, in response to falling nutrient levels.

Throughout the development of the phytoplankton and bacterial populations, there was an accompanying increase in the P.O.C. levels in the mesocosm (fig.21) which reached a maximum ($1.4mg.l^{-1}$) on day 6. Parsons and Strickland (1962), Barber (1966), Anderson and Zeutschel (1970) and Biddanda (1985) show that P.O.M. synthesis is dependant on the presence of bacteria and occurs at the expense of D.O.C. in the water column. During the decline of the bacterial population between days 6 and 10, the P.O.C. levels also fell, suggesting that following the decline of D.O.C. concentrations, active mineralisation of particulates took place as described by Fukami et al. (1985a, b); this is initially rapid, and results in the mineralisation of labile fractions of the P.O.M.

There were marked changes in the taxonomic structure and physiological properties of the plateable bacterial populations throughout the development and decline of the assemblage. The initial population was highly diverse, as shown by all three measures of diversity (D, H', E - see fig.33) which is typical of populations in upwelled water (Section 2). This assemblage had a high proportion of fermentative isolates (fig.30) and an H' = 3.7, which was comparable to that recorded by Martin and Bianchi (1980) in oligotrophic temperate waters. With the increase in numbers and biomass following phytoplankton bloom development, there was an initial increase in diversity which peaked on day 3 during midlog phase of the phytoplankton increase: this was accompanied by a drop in the number of fermentative isolates recorded, with Pseudomonas and Acinetobacter isolates becoming dominant in the assemblage. At the peak in bacterial numbers and biomass, the diversity was very low and the plateable population was dominated by Pseudomonas isolates. This process bears a strong resemblance to the development of bacterial blooms in other systems. Fukami et al. (1985a, b) noted similar decreases in population diversity and in the occurrence of fermentative isolates associated with increases in numbers, biomass and plateability of bacteria during the decay of phytoplankton detritus, while Martin and Bianchi (1980) and Martin (1980) noted marked reductions in diversity and

occurrence of fermentative isolates in bacterial populations associated with natural phytoplankton blooms. Kaneko et al. (1977) found an inverse relationship between biomass and species diversity in the Beaufort Sea, in confirmation of the hypothesis of Margalef (1951 et seq.) that oligotrophic communities respond to high nutrients by developing populations which consist of relatively few well adapted and opportunistic species. This population showed a similar trend: as diversity decreased, there was an increase in the number of isolates able to hydrolyse casein, gelatin, agar and C.M.C. (fig.28).

As both the phytoplankton and bacterial populations declined, there was an increase in the diversity measures H', D and E, which was reflected in an increased taxonomic diversity: both fermentative isolates (Vibrio and Enterobacteriaceae) were again present in the assemblage, but there was a decrease in the number of isolates able to hydrolyse milk, gelatin, agar and C.M.C. The first part of the incubation conformed closely to bacterial population development associated with phytoplankton bloom development described by various authors for both natural (Bell and Kuparinen, 1984; Delattre et al., 1979; Martin, 1980; Martin and Bianchi, 1980; Kattner et al., 1983; Barlow, 1982a, b, 1984) and en- closed systems (Linley et al., 1981; Laanbroek et al., 1985 ;

Newell et al., 1980, 1981; Jahnke et al., 1983; Fukami et al ., 1985a, b; Laake et al., 1983b), in which bacterial population development was linked to the provision of abundant, labile nutrient either in the form of D.O.C. or detrital material.

During the second part of the incubation, however, no discernable increase in phytoplankton abundance was found, nor were there any concommittant increases in D.O.C. or P.O.C. Nonetheless, after a short lag period (days 8 - 10) a second increase in bacterial numbers and biomass commenced, which persisted to day 18, whereafter both numbers and biomass remained stable till day 30 (figs 22, 23). Both numbers and biomass of bacteria were higher during this period than they had been during the first part of the incubation.

Examination of the D.O.C. and P.O.C. concentrations (figs 20,21) suggest that continued mineralisation of these resources continued during this period. Up to day 12, rapid mineralisation ($0.17 mg.\ell^{-1}.d^{-1}$) of the P.O.C. occurred, but thereafter a slow ($0.0042 mg.\ell^{-1}.d^{-1}$) utilisation continued till the end of the incubation. The P.O.C. levels (fig.21) while somewhat erratic, ranged between 1.0 and $3.0 mg.\ell^{-1}$ between days 10 and 18; thereafter they fell precipitously and remained low (between 0.0 and

1.5mg.ℓ^{-1}) for the duration of the enclosure. These results suggest that during the development of this population, cells were dependent on the mineralisation of recalcitrant D.O.C. and P.O.C remaining from the initial period.

It is known that not all D.O.C. released by phytoplankton is immediately utilisable: Eberlein et al. (1983) showed that, together with glucose and easily utilisable D.F.A. A., photosynthetically derived organic carbon (P.D.O.C.) contained recalcitrant compounds which required biological modification before they could be utilised by bacteria and suggested that they may remain unutilised until bacterial successions occurred which produce populations able to use them. Fukami et al. (1985a, b) suggest, on the basis of studies of the decay of Skeletonema costatum detritus, that at least two distinct populations of bacteria are involved in mineralisation of detritus - the first dependent on easily utilisable D.O.C. and particu lates and the second dependant on recalcitrant compounds, while Itturiaga and Hoppe (1977) and Itturiaga (1979) showed that during the mineralisation of sedimenting phytoplankton debris, the small molecular weight D.O.C. and P.O.C. components were utilised earlier, than larger molecular weight recalcitrant compounds.

It was evident that the increase in numbers and biomass

occurred in all size classes, but was particularly marked in the large rods and small cocci. It is probable that a fragmentation response continued throughout this period amongst those strains unable to utilise the remaining D.O.C. and P.O.C., in concert with the development of a new and active assemblage, leading to an increase in small cocci, together with an increase in the larger size classes. The increase in biomass was attributed chiefly to the increase in numbers of large rods, which finally composed nearly 80% of the total biomass by day 30 (fig.24b). A similar increase in large rods during the late stages of degradation of phytoplankton debris was noted by Linley et al. (1981).

Changes in the composition of the plateable population accompanied the increase in numbers and biomass. During early exponential growth (days 11 - 16) there was an increase in diversity (figs 33a-c) as had occurred between days 1 and 3. An increase in the number of genera was recorded, as a population comprising Vibrio, Enterobacter, Cytophaga/Flavobacterium, Acinetobacter and Pseudomonas replaced the lag phase population which had been dominated by Pseudomonas. Throughout this period, chromogenic strains formed an increasingly large part of the plateable population, whether assessed by plate count (fig.29) or random selection (fig.30).

As the population number and biomass peaked, there was a decrease in diversity on all indices (H', D, E) and the number of genera decreased as fermentative strains disappeared from the assemblage. As had occurred on day 5, the plateable population was dominated by Pseudomonas strains, with Acinetobacter also being represented, together with a high percentage of chromogenic strains (Cytophaga/Flavobacterium).

Results of radiolabelled glucose and amino acid uptake experiments suggest that this population was unable to take up D.O.C. in the form supplied, and despite rapid growth, no increase in uptake activity occurred after day 10. Furthermore, few of the isolates displayed a wide range of hydrolytic properties: an increase in the number of agarase producing isolates did occur, but very few were able to hydrolyse casein, gelatin or C.M.C. This population was thus clearly distinguished from the highly active population of day 5, which readily took up D.O.C. and showed a wide range of hydrolytic properties. In addition, although there was a slight increase in the viable count, the percentage plateability remained low.

From day 30, the number and biomass of cells fell, and continued to do so until the termination of the enclosure on day 43. This was accompanied by an increase in

generic diversity as fermentative isolates again appeared in the assemblage. By day 43 numbers, biomass and the generic distribution resembled those of the first 3 days, but this increase in diversity is reflected in only one diversity index (D) - both H' and E fail to show any increase in diversity, largely due to the paucity of isolates showing hydrolytic activity.

A seemingly paradoxical feature of the terminal stages of the enclosure was the increase in %P (fig.26). It is suggested that this reflects a maintenance of competent nutritional mechanisms during the fragmentation phase, and a similar phenomenon has been noted during starvation of purified isolates by Amy and Morita (1983a, b) and Amy et al. (1983). In this instance, an increase in the viable count occurred between days 25 and 33, as the total count began to decrease. The increase in %P resulted both from this increase in the viable count and from the progressive drop in the total count with the onset of oligotrophic conditions.

There were thus two distinct bacterial populations associated with the development of the phytoplankton bloom in this experiment. This differed from reports by Linley (1983), Linley et al. (1981) and Fukami et al. (1985a, b) on the mineralisation of phytoplankton and zooplankton detritus and kelp exudates, in which single bacterial

blooms occurred. Fukami et al. (1985a, b) suggested, however, that two populations were involved, the first dependant on labile compounds and the second on refractory compounds, but their development was synchronous and no bimodal peaks in numbers and biomass occurred. Newell (1982, 1983) however, showed that during the decay of saltmarsh plant detritus there was an initial (10 day) bacterial bloom associated with labile D.O.C. and P.O.C. mineralisation, which was replaced by a second bloom associated with the refractory particulates.

The development and decline of both populations conformed to descriptions by Margalef (1967, 1968, 1979), Martin and Bianchi (1980) and Martin (1980); periods of high numbers and biomass were characterised by populations of low diversity, while highly diverse populations were associated with periods of low numbers and biomass.

Multivariate analysis of the two populations showed that they differed considerably. Diverse populations associated with early log phase development in both instances (days 3, 16) were similar to one another (fig.32) and fairly similar to the terminal population, but populations associated with the first (days 5, 7) and second (days 28, 34) population peaks bore little resemblance to one another, beyond a similarity in generic composition (fig. 30). The first population,

associated with the phytoplankton bloom, was highly active in taking up D.O.C. and displayed a wide range of hydrolytic properties, while the second population did not take up radiolabelled D.O.C. and had a restricted range of hydrolytic properties.

These results have considerable implications for future studies. At present, most estimates of bacterial activity and productivity are based on uptakes of radiolabelled glucose (Wright and Hobbie, 1966, 1967; Vaccaro and Jannasch, 1966; Takahashi and Ichimura, 1971; Novitsky, 1983), amino acids (Bright and Fletcher, 1983a, b; Novitsky, 1983; Simon, 1985) or tritiated thymidine (Fuhrman and Azam, 1980; Ducklow et al., 1982; Kirchmann et al., 1982; Lovell and Kanopka, 1985a, b). As shown here, however, these estimates will only include those bacteria or populations dependent on labile D.O.C. or P.O.C., and only estimates based on number or biomass increases will include both populations. Furthermore, bacterial populations cannot be regarded as homogeneous units - changes in productivity, rates of uptake and properties have to take into account that successive samplings may measure properties of different bacterial groups involved in an orderly succession, determined by nutrient type and availability.

SECTION 4: BACTERIAL POPULATIONS AND HETEROTROPHIC ACTIVITY ASSOCIATED WITH FRONTAL SYSTEMS IN THE BENGUELA REGION.

4.1 General Introduction

The southern portion of the Benguela region is an area of great hydrological complexity, characterised by the convergence of the Agulhas current and South Atlantic Surface Water with upwelled South Atlantic Central Water.

During the summer months, the persistence of offshore south - easterly winds and consequent active upwelling leads to the formation of a relatively stable oceanic front which extends from Cape Agulhas (35°S) to Cape Columbine (33°S) (fig.1). The exact position and hydrological characteristics of the front varies considerably, depending largely on prevailing wind patterns, but in general the thermal front follows the shelf break from Cape Columbine to Cape Point. The intensity of the front is variable: although it is usually present at around 1°C per nautical mile, it may intensify to a gradient of several degrees over a few hundred metres. Maximum gradients usually occur from mid-summer to mid-autumn, when the thermal front is enhanced by convergence of the cold upwelled water with warm oceanic water, but the surface features of the front alter rapidly when the south easterly winds drop or onshore winds commence, and oceanic waters move inshore or flood over and mix with the upwelled water. (Andrews and Hutchings, 1980; Lutjeharms, 1981; Hutchings et al, 1984, 1985; Shannon, 1985b).

The surface waters offshore of the front are derived from South Atlantic Surface Water, Agulhas Current Water or Agulhas Bank Water (Hutchings et al., 1985). In general these are poor in nutrients and carry a low biomass of phytoplankton and zooplankton. Newly upwelled water on the other hand, while presenting a low biota, is rich in nutrients such as NO_3, SiO_3 and PO_4. Although low in D.O.C. and P.O.C. at source, by the time the upwelled water has passed through inshore regions such as the kelp-beds, it may also carry a high D.O.C. and P.O.C. load derived from the inshore macrophytes and benthic sediments.

Once advected to the surface, such upwelled water is subject to blooms of phytoplankton, bacteria and zooplankton, which rapidly deplete the nutrients (see Sections 2, 3). As a result, aged upwelled water abutting on the front is frequently nutrient poor and the front is seldom marked by distinct nutriclines, although marked changes in phytoplankton, zooplankton and ichthyoplankton may be noted across it (Hutchings et al., 1985).

The frontal system is an area of convergence, marked by strong salinity and temperature gradients. These generate conditions of varying stability, best described in terms of σ^t values, an expression of water density

independent of pressure but dependant on the effects of temperature and salinity. Usually, two zones of relative stability occur: the pycnocline, at which there are rapid vertical changes in σ^t values; and the front, in which rapid horizontal changes in ^{t} arise.

Several authors (Owen, 1981; Dunson and Ehlert, 1971) suggest that the primary biological effect of the front and the pycnocline lies in their provision of an area of relative stability, at which accumulative processes take place. However, since the pycnocline also marks a barrier between nutrient rich bottom and nutrient poor surface water, periods of instability in this boundary layer are necessary to maintain biological activity, since prolonged periods of stability lead to the depletion of nutrients (Eppley et al., 1973; Eppley and Peterson, 1979; Legendre, 1981) and the sinking from the euphotic zone of phytoplankton cells (Eppley et al., 1967; Loder and Platt, 1985). In such instances, there are indications that centres of biological activity shift from the shallower water to the pycnocline and that biological activity changes from a system dominated by photosynthetic processes to one centred on the remineralisation of particulate organic matter (Painting et al., 1985; Lucas, 1985).

The alternative situation, where extensive and continuous mixing occurs, will also result in lowered biological activity, since phytoplankton cells fail to spend an optimum period of time in the euphotic zone (Loder and Platt, 1985). Enhanced biological activity at the front may arise from the steep or vertical stratification, which offers an optimum balance between mixing and stability (Owen, 1981) as well as acting as an area of physical accumulation of particles and biomass (Floodgate et al., 1981; Lutjeharms, 1981; Owen, 1981).

The biological features of fronts have been extensively investigated. Differences between water masses on either side of the front, and between surface and bottom waters may be so great as to suggest that they constitute separate ecosystems. Fogg et al. (1985a, b) working in the Irish Sea on a tidally mixed front, showed that water masses associated with the front could be statistically distinguished on the basis of both physical and biological characteristics. Similarly, Holligan et al. (1984 a, b) note that each of the three water masses associated with the Ushant Front had a characteristic phytoplankton community associated with it.

In tidally mixed frontal systems, such as the West English Channel, the West Irish Sea and the Celtic Sea, it has been shown that phytoplankton activity was limited on the horizontally stratified side of the front by sinking of the phytoplankton cells to the pycnocline, at which depth photosynthesis was light limited, while at the front itself, vertical stratification led to the localisation of cells within the euphotic zone, resulting in elevated levels of photosynthesis (Fogg et al., 1985b; Turley and Lochte, 1985; Holligan et al., 1984b). Indeed, elevated levels of Chl a are often considered to be characteristic of frontal zones and have been described for European (Pingree et al., 1974, 1977; Savidge, 1976; Savidge and Foster, 1978), South African (Andrews and Hutchings, 1980; Hutchings et al., 1984, 1985) and Pacific waters (Eppley et al., 1973).

The associations between heterotrophic bacterial activity and photosynthesis are now well established (Cole et al., 1982; Joint and Pomroy, 1982; Lancelot, 1979, 1984; Riemann et al., 1984; Lucas et al., 1985) and appear to be most closely linked to photosynthetically derived dissolved organic carbon (P.D.O.C.) in the euphotic zone (see Sections 2, 3). Furthermore, the close association between bacteria and particulate organic matter is also well established; as the front

is often an area of elevated levels of both photosynthesis and particle accumulation (Hutchings et al., 1985), bacterial numbers, biomass and activity might reasonably be expected to be elevated at a front.

In such a system, the inter-relationship of bacteria, phytoplankton, zooplankton and bactivory by ciliates and flagellates (Fenchel, 1982; Newell et al., 1985; Lochte and Turley, 1985) might contribute significantly to the recycling of nutrients and even in fronts showing poor nutriclines, significant plankton communities may thus be maintained (Linley et al., 1983; Holligan et al., 1984b; Hutchings et al., 1985; Probyn, 1985). Some controversy surrounds this question however, as there is evidence that bacteria may act as a carbon sink, and that recycling of bacterial carbon by flagellate predation is restricted (Ducklow et al., 1986).

Floodgate et al. (1981) showed that at a front in Liverpool Bay, bacterial mineralisation of accumulated detritus led to increased nutrient levels which stimulated phytoplankton growth, and Hanson et al. (1983) and Hanson and Lowery (1985) showed that in the Southern Oceans horizontal distributions of microbial biomass corresponded to the position of the frontal zone in the Drake passage, with microbial populations three times higher in this zone than in the adjacent oceanic waters. Furthermore, they showed that microbial biomass

and production were two to three times higher than in the adjacent waters, features which they attributed to both mixing (stratification) effects and mechanical accumulation of biomass. At the Ushant front in the English Channel, Holligan et al. (1984a) and Linley et al. (1983) have shown that heterotrophic bacteria comprise 10-30% of the standing stock, with the highest biomass of bacteria occurring in the euphotic zone of the front itself, with much lower levels occurring on either side of the front. This situation is not invariable, however; and enhanced biological effects, while associated with the frontal structure, may occur at some distance from the front itself. In a tidally mixed front, Lochte and Turley (1985) found that increased microbial activity was associated with stratified surface waters adjacent to the front although the highest cell specific uptake activity was noted at the front itself, while Pingree et al. (1974, 1977) recorded dinoflagellate blooms remote from a front but caused by release of nutrients from it.

Extensive sampling of the hydrological, nutrient, phytoplanktonic, zooplanktonic and ichthyoplanktonic characteristics of the Benguela Oceanic front have been done (Andrews and Cram, 1969; Andrews and Hutchings 1980, Hutchings et al., 1984, 1985; Shannon, 1985a, b), some of which have shown marked changes across the

front. Strong gradients in Chl a are shown to occur (Andrews and Hutchings, 1980; Hutchings et al., 1984, 1985) as well as secondary peaks in NO_3, SiO_3 and PO_4, but there has been little evidence of enhanced zooplankton or microplankton standing stocks at the front itself, although substantial differences in the structure of the offshore and inshore populations do occur. The size of the inshore populations tends to be much higher, and the biota is limited to the inshore by the frontal features. Distributions may thus be compressed by an inshore flooding of warm oceanic water. Mesozooplankton stocks, however, appear to be very sensitive to frontal features, and marked enhancements of standing stock have been noted, particularly at upwelling fronts (Hutchings et al., 1985). Species composition of zooplankton communities does however change across the front, and distinct warm water, thermocline and cool water communities have been shown to occur (Hutchings, 1979).

At present, no studies have been conducted on the effect of the front on bacterial populations of the Benguela current. This section presents the results of two investigations conducted in March, 1983 and December, 1984. The first survey, conducted off the Cape Peninsula coast, served as a preliminary investigation of horizontal and vertical distribution of bacteria and

bacterial properties, relative to hydrological conditions. The second, conducted off the coast at Cape Columbine, was a more intensive investigation examining the effect of changes in hydrological conditions on the heterotrophic bacterial populations.

SECTION 4.2: CHANGES IN BACTERIAL POPULATIONS AND ACTIVITY ALONG A TRANSECT CROSSING THE BENGUELA OCEANIC FRONT IN THE CAPE PENINSULA REGION

4.2.1 Introduction

The upwelling monitoring line (U.M.L.) surveyed during this cruise was established by the Sea Fisheries Research Institute, Cape Town, in 1970 and has been surveyed 45 times to date. These surveys are necessarily brief, since they attempt to obtain an *instanta* picture of hydrographic conditions uninfluenced by major changes in conditions during the survey. Previous surveys have provided information on the physical attributes, nutrient and Chl a concentrations, phytoplankton, zooplankton and ichthyoplankton community composition and dynamics, and explored changes in these parameters both spatially (along the U.M.L. and particularly at the frontal boundary) and seasonally. (Andrews and Hutchings, 1980; Hutchings et al 1984, 1985; Olivieri *et al*., 1985). The work described here was the first to provide information on the bacterioplankton along the U.M.L. and at the frontal zone.

4.2.2 Methods and Materials

4.2.2.1 Sampling site and sampling procedure

This survey was conducted in the summer of 1983 (21.03.83) from the R.S. Africana. The Upwelling Monitoring Line (U.M.L.) consists of ten stations lying

off the West coast of the Cape Peninsula at distances of 5.5, 13.5, 33.0, 52.0, 61.0, 80.0, 98.0 and 114.5km respectively from the shore. The line initially runs in a North- Westerly direction (315°) but turns to the West (295°) 60km offshore to cross the oceanic front, which usually lies between 60km and 80km offshore (fig.1). The line thus approximates the direction taken by water upwelling off the Cape Peninsula (Hutchings et al., 1984).

At each station, continuous temperature and density (C.T.D.) profiles were recorded from the surface to the bottom of the water column, and water samples were obtained in 30ℓ N.I.O. bottles on a rosette sampler, triggered at twelve depths determined by the 100%, 50%, 25%, 1% and 0.1% light levels, and by the position of the pynocline and the bottom. These water samples were used for all nutrient and biological analyses described hereafter.

4.2.2.2 Hydrology

Hydrological features of the U.M.L. were determined from the C.T.D. profiles of salinity, temperature and density (σ^t). Wind stress vectors have been previously reported (Hutchings et al., 1984).

4.2.2.3 Nutrients

Samples were analysed for NO_3, SiO_3 and PO_4. These were preliferated through 25mm Whatman GF/F filters. The filtrate was stored frozen prior to analysis in the laboratory, which was carried out using a Technicon Nutrient Analyser following the methods of Mostert (1983).

4.2.2.4 Chlorophyll a

2ℓ samples of seawater were filtered through 47mm Whatman GF/F filters at each sampling station. The filters were stored frozen (-4.0°C) prior to solvent extraction of Chl a, which was analysed spectrophotometrically as described by Brown (1984).

4.2.2.5 Bacterial Numbers and Biomass

At each sampling station, 10mℓ water samples were fixed with Analar glutaraldehyde (2.0% v.v final concentration) and stored at 4°C in the dark until final analysis in the laboratory. A.O.D.C. and computation of total numbers and biomass was done following methods described in Sections 2.2.3 and 2.2.4.

4.2.2.6 Viable count and percentage plateability

The viable count ($CFU.ml^{-1}$) was determined at stations 13.5, 33.0, 52.0, 80.0, 98.0 and 114.5km offshore. At each station, the viable count was determined from the 100% and 25% light level, at the pycnocline and from 20m below the pycnocline. Quadruplicate 0.1 mℓ aliquots were plated onto 0.5% Pep-SWA plates (see Appendix). Plates were counted following methods described in Section 2.2.5.

4.2.2.7 Characterization of bacterial populations

The plateable bacterial populations at the 100% and 25% light levels and at the pycnocline were characterised to generic level at stations 8 (80.0km), 9 (98.0km) and 10 (114.5km), using the random colony selection method described in section 2.

4.2.3 Results

4.2.3.1 Wind

The progressive wind stress vector diagram for the period 08.02.83 to 31.03.82 is shown in fig.34. Almost throughout this period, south-easterly winds prevailed, but immediately prior to sampling along the U.M.L. a wind reversal to north-westerly occurred.

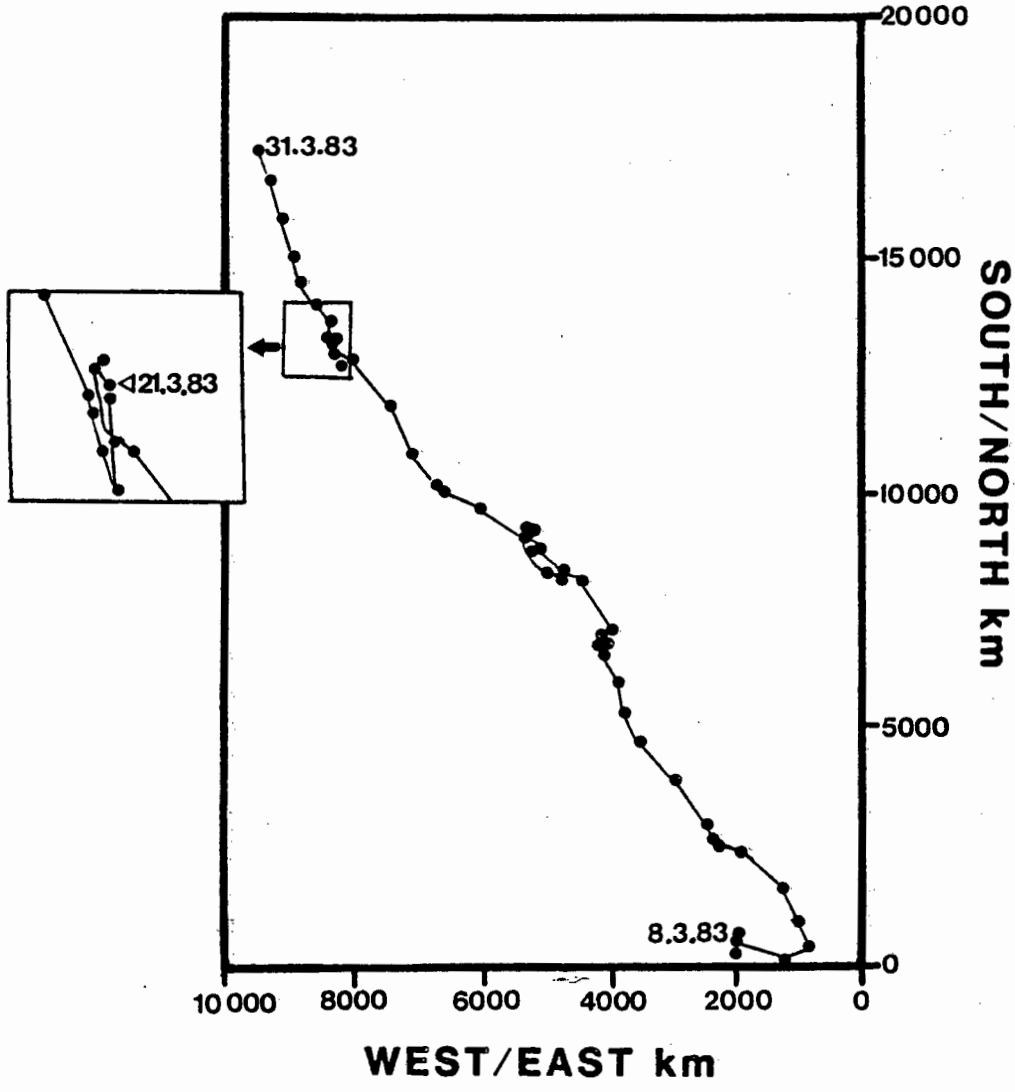

FIGURE 34: Progressive wind-stress vector diagram for the period 09/03/83 - 31/03/83. The U.M.L. was sampled on 21/03/83. (From Hutchings et al., 1984).

4.2.3.2 Temperature

Results of temperature sections are shown in fig.35a (to 350m) and b (detail to 90m). These show that there was little thermal evidence of active upwelling, and that the cold (<11.0°C) water normally associated with upwelling lay below 15m even at the inshore stations. With the exception of Station 10 (114.5km), all surface stations showed temperatures of 13-15°C. Between stations 9 and 10 a pronounced thermal front occurred (15.0° - 19.9°C in 5km) with vertical isothermals extending to 80m depth. Inshore of this front, stratification was not marked, nor was a pronounced thermocline anywhere evident. Between 25km and 75km offshore, a large patch of warmer (>15.4°C) water was evident, in the upper 5m of the water column, which gave rise to minor thermal fronts at its outer boundaries. This patch probably arose as a result of sunwarming.

4.2.3.3 Salinity

Results of salinity determinations are shown in fig.36. These show the complex interaction of at least 5 bodies of water, which can be characterised on the basis of their salinity.

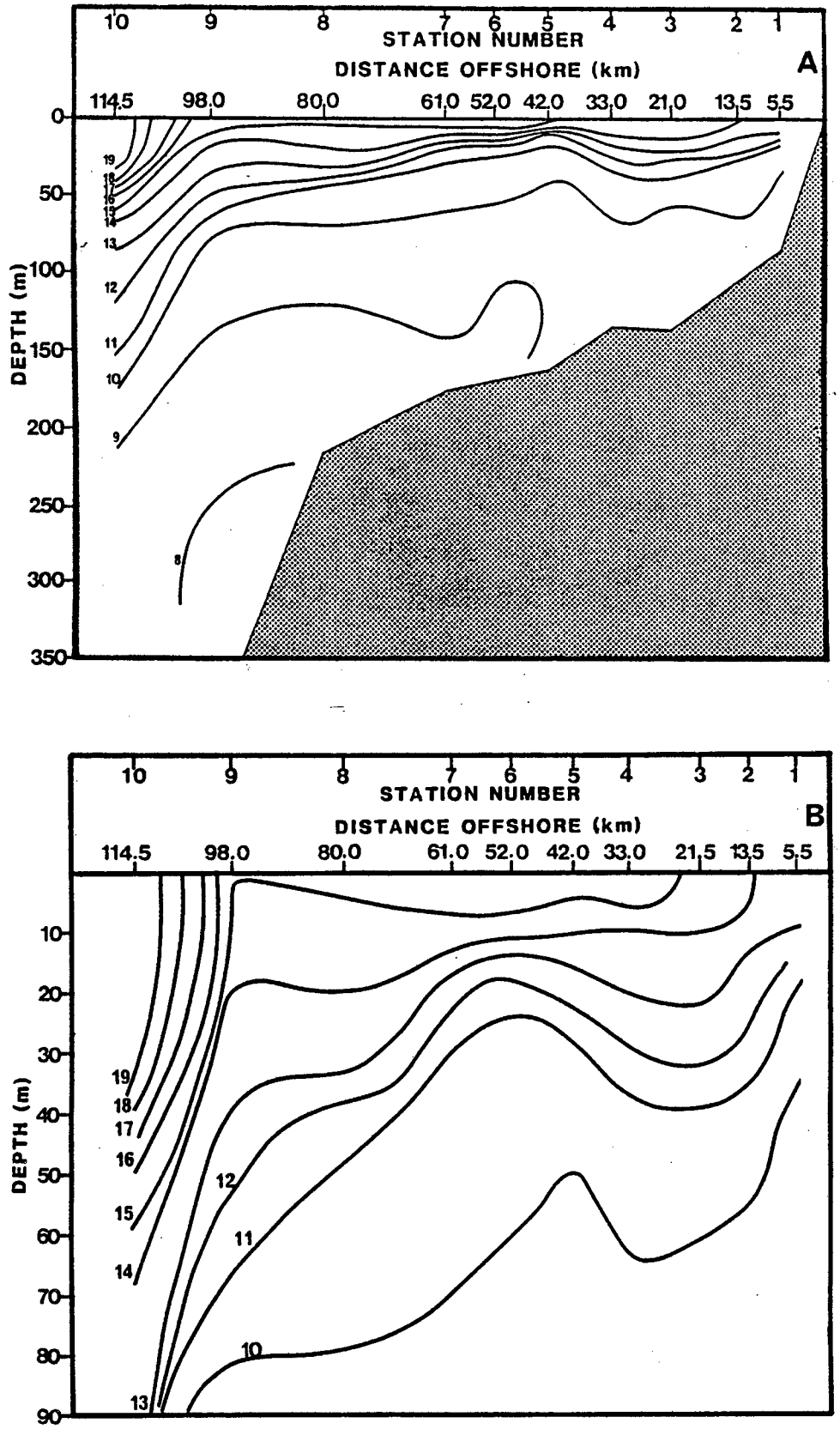

FIGURE 35: Profile of isotherms (°C) along the U.M.L. (Fig. 35a: Bottom topography and temperature profile to 350m. Fig. 35b: Temperature profile to 90m).

Underlying the monitoring line was a body of low salinity (<34.82°/oo) water, identified as South Atlantic Central Water. At the inshore stations (up to 21.5km offshore), this water intruded to the surface, bounded by clear vertical isohalines to 60m depth in a pattern typical of upwelling water. Adjacent to this narrow area of upwelling water, a patch of higher salinity water extended to 98km offshore, to a depth of 50m, with a salinity of >34.86°/oo, forming a wedge shaped body of water. Contained within this body of water, at a distance of 70km offshore, was a small patch of low salinity water (<34.80°/oo) extending to 20m depth, identified as aged upwelled water. Since the general direction of flow of such water is north-westerly, (Hutchings, 1984) this water probably originated far south down the Cape Peninsula. Adjacent to this low salinity water was a second plume of more recently upwelled water (<34.90°/oo) probably originating closer to the site of the U.M.L.

Between stations 9 and 10 a pronounced salinity front occurred, corresponding to the position of the thermal front and marking the convergence of the high salinity oceanic water (>35.0°/oo) with the lower salinity inshore Benguela waters.

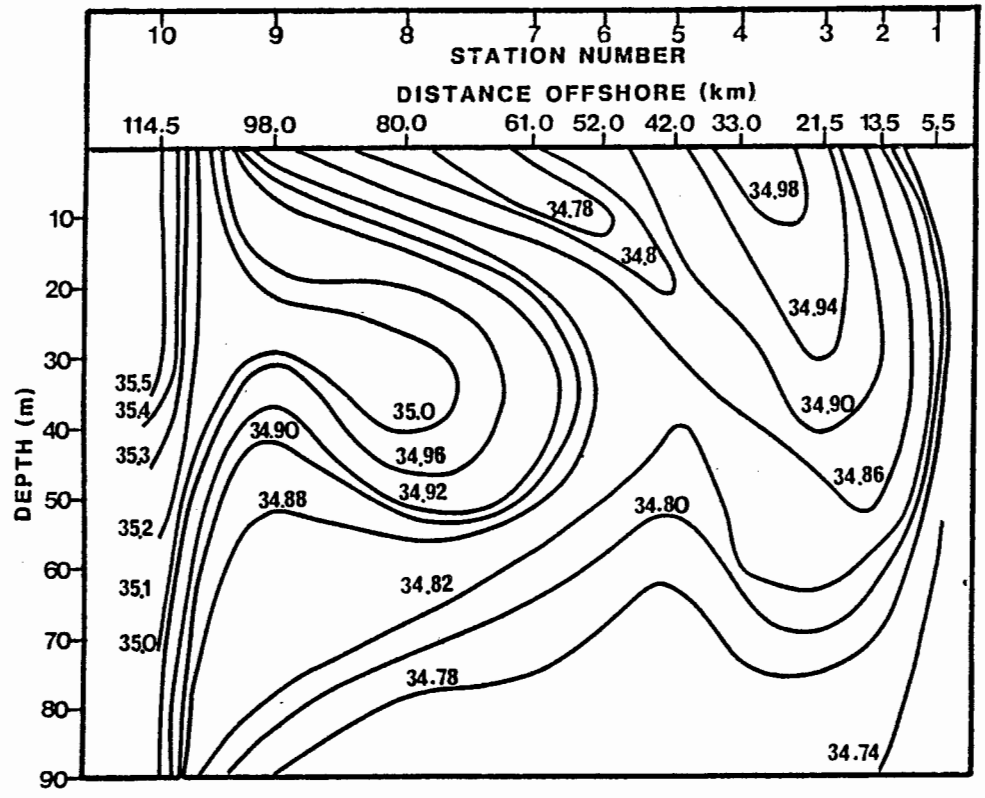

FIGURE 36: Profile of salinity isohalines ($^{o}/oo$) along the U.M.L.

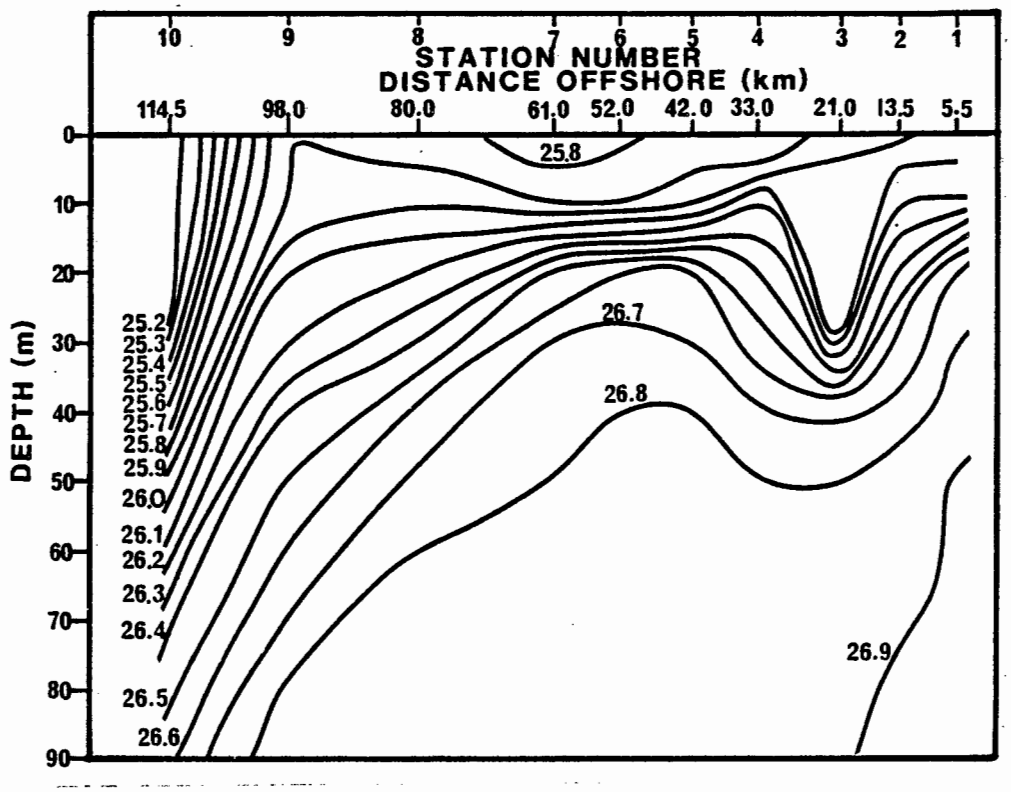

FIGURE 37: Profile of density (σ^{t}) isopycnels along the U.M.L.

A subsurface tongue of high salinity water (35.0‰) occurred at a depth of 30m between Station 8 (80.0km) and 9 (98km). This was either a subsurface intrusion of oceanic water resulting from the wind reversal or, more likely, an intrusion of Agulhas Bank Water which had been conducted North into the Benguela system during the persistent south east winds which had previously occurred.

4.2.3.4 Density

The σ^t profiles (fig.37) corresponded very closely to the temperature profiles, again showing a marked front between station 9 and 10. In general the pycnocline was not distinct, but stratification was fairly intense at stations closer inshore than 70km. Strong downturns of the isopycnels occurred at the front itself and at station 2 (13.5km) and 4 (33,0km). These suggest that vertical water movement occurred at the front itself, and at station 4 on the offshore side of the upwelling plume.

4.2.3.5 Nutrients

Nutrient profiles for NO_3, PO_4 and SiO_3 are presented in figs 38, 39 and 40. In general, these show an increase in nutrient concentration with depth, and a

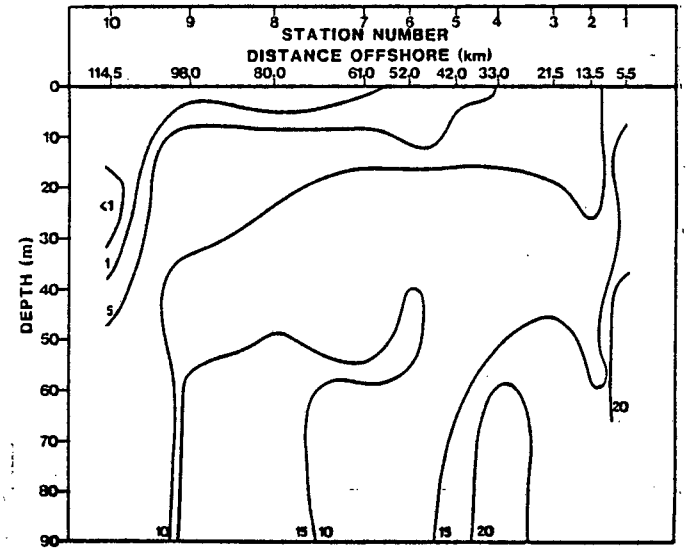

FIGURE 38: Profile of NO_3-N isopleths (µg at NO_3-N.l^{-1}) along the U.M.L.

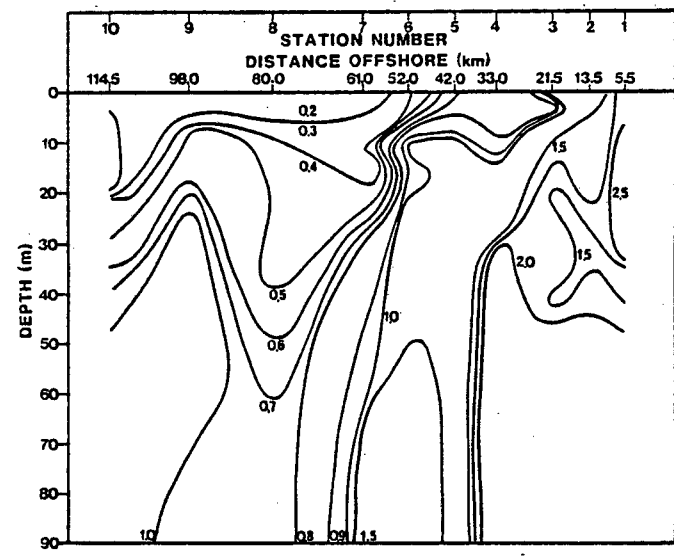

FIGURE 39: Profile of PO_4 isopleths (µg at.l^{-1}) along the U.M.L.

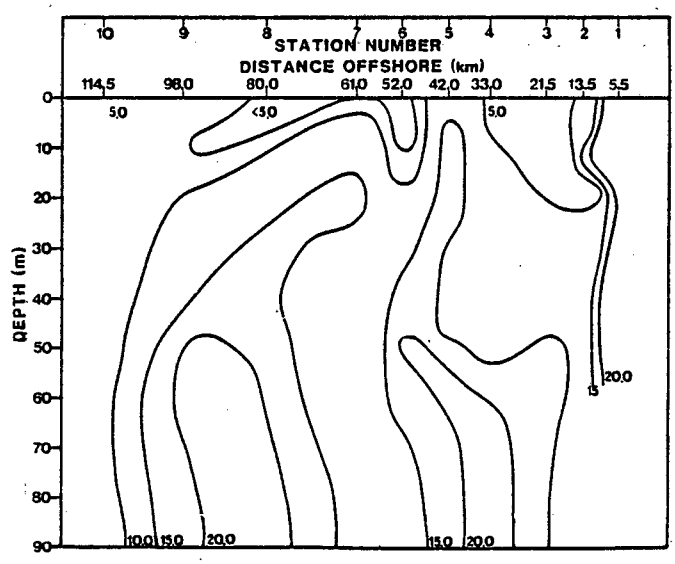

FIGURE 40: Profile of SiO_3 isopleths (µg at.l^{-1}) along the U.M.L.

decrease in concentration with increasing distance from the shore. The deep South Atlantic Central Water was particularly rich in NO_3 (>20.0µg at NO_3-N.l^{-1}) and PO_4 (>1.5µg at.l^{-1}) and recently upwelled water close to the coast was correspondingly high in nutrients as described by Andrews and Hutchings (1980), Barlow, (1982a, b) and Shannon (1985b).

Oceanic waters were low in nutrients, although in this case, neither the NO_3 concentration (1-2µg at NO^3-N.l^{-1}) nor the PO_4 concentration (0.2µg at .l^{-1}) could be considered limiting to phytoplankton growth (Barlow, 1984). These are not the only limiting nutrients however, and impoverishment of micronutrients not measured here may play an important role in limiting phytoplankton growth (Brown, 1982).

4.2.3.6 Chlorophyll a

Profiles of Chl a distribution are presented in fig.41, and horizontal distributions at specified depths in fig.44. Chl a concentrations throughout the U.M.L. were low (<10.0mg.m^{-3}) although within the range (1.0 - 20.0mg.m^{-3}) given for this area by Andrews and Hutchings (1980) (fig.41). Areas sharing concentrations >1.0mg.m^{-3} were largely limited to the euphotic zone within 90km of the shore, and their distribution closely

paralleled the distribution of water of salinity <34.98°/oo, suggesting that they were limited to upwelled waters. The highest concentration of Chl a (9.53mg.m^{-3}) was present at the first station, (5.5km) where upwelling had recently taken place. A second patch of high Chl a concentration (>4.0 mg.m^{-3}) was present to 10m depth between 40km and 65 km offshore, that is between the centres of the two aged upwelling plumes previously described. A third bloom, of a much smaller Chl a concentration (1.07 - 1.19 mg.m^{-3}) occurred on the oceanic side as a subsurface maximum extending between 15m and 42m depth.

4.2.3.7 Bacterial numbers and biomass

The distributions of bacterial numbers and biomass are presented in figs 42 and 43. Overall, numbers and biomass of bacteria tended to decrease with depth, with a slight increase frequently occurring at the pycnocline. Above the pycnocline, numbers tended to exceed 1.0 x 10^6 cells.ml^{-1}, falling as low as 0.29 x 10^6 cells.ml^{-1} below the pycnocline. Above 30m depth, the distribution was patchy and some samples with high cell densities were present. A small patch (>4.0 x 10^6 cells.ml^{-1}) occurred at Station 1, but this was not reflected in an increase in biomass, since the bulk of these cells were small cocci. This suggests that the

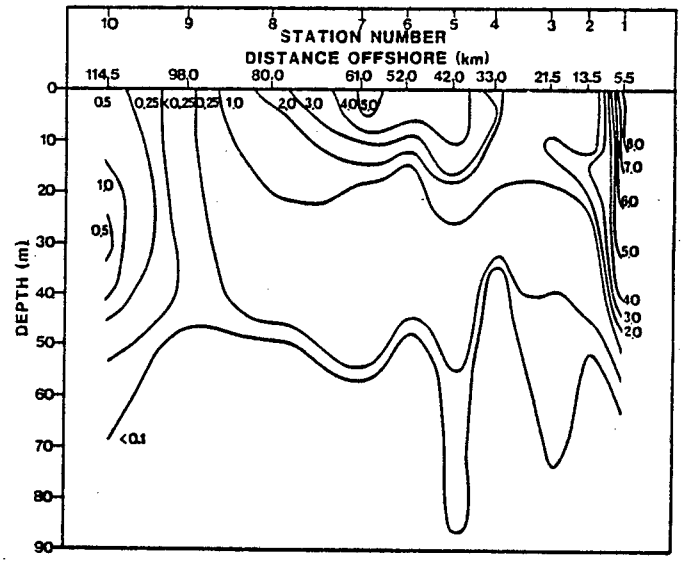

FIGURE 41: Profile of the distribution of chlorophyll a (mg. m^{-3}) along the U.M.L.

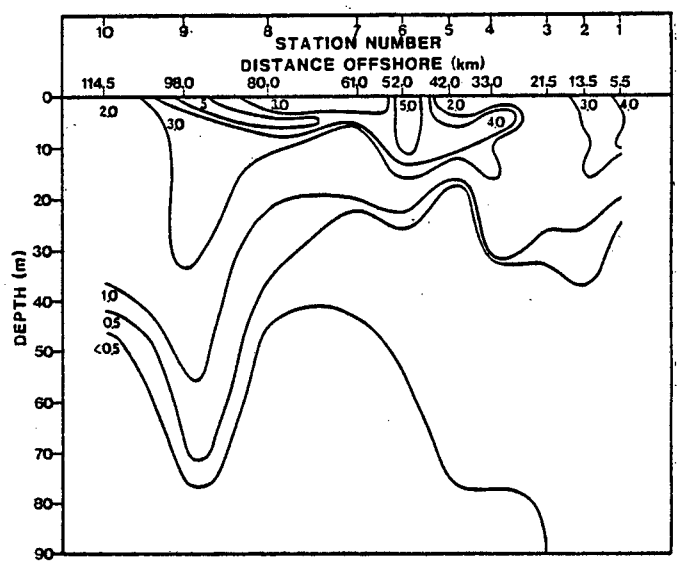

FIGURE 42: Profile of the numerical distribution of bacteria (cells x $10^6.ml^{-1}$) along the U.M.L.

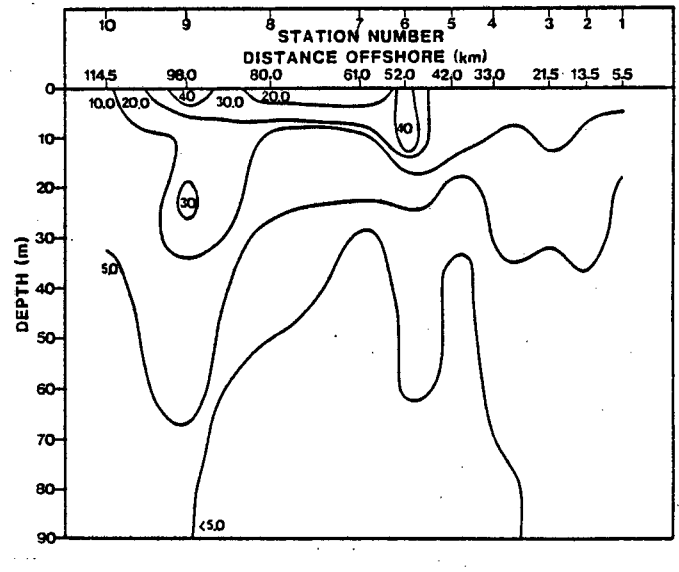

FIGURE 43: Profile of the distribution of bacterial biomass (µg $C.l^{-1}$) along the U.M.L.

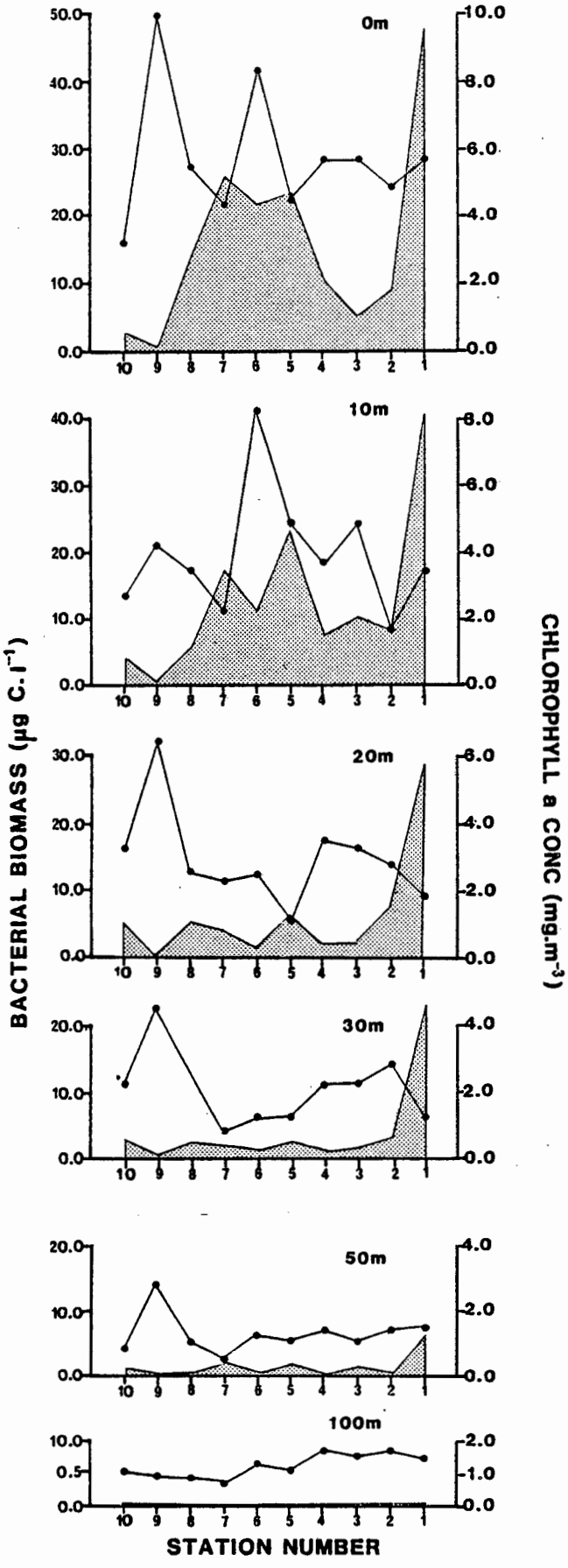

FIGURE 44: Changes in the horizontal distribution of Chla ($mg.m^{-3}$ - ▒) and bacterial biomass (µg $C.l^{-1}$ - —●—) at specified depths along the U.M.L.

population was of recently upwelled origin. At the surface, both numbers and biomass tended to be similar up to Station 5, but a large patch broke surface at Station 6, extending through the subsurface waters to 5m at Station 4 and to the surface at Station 9. The outer boundary of this patch lay against the front and it is interesting to note that in this region it penetrated to 50m depth. A close correspondence was noted between the distribution of this patch and the course of the 15.0°C isotherm (fig.35) suggesting that in the region of the front, downwelling had affected the bacterial distribution. A graph of biomass along specific depth transects clearly demonstrates the presence of the patches at the central stations and at Station 9, to a depth of 50m (fig 44).

Overall, a correlation was noted between the biomass of bacteria and the concentration of Chl a ($a = 1.60$, $b = 11.56$, $r = 0.34$, $p>.001$ $<.01$ for the equation $y = ax + b$, where y = biomass ($\mu gC.l^{-1}$) x = Chl a concentration ($mg.m^{-3}$)), but this was not invariable, and it is interesting to note that in some areas of high Chl a, (such as Station 7, Station 5 and Station 1) bacterial numbers and biomass were low (fig.44).

4.2.3.8 Viable count and percentage plateability

Results of analyses of viable count (C.F.U. ml^{-1}) and %P are presented in figs 45a and b. Throughout the transect, both the viable count and percentage plateability were low. This is a common feature of marine waters as noted in Section 2. Above the pycnocline, there was a marked increase in the viable count from Station 2 to Station 4, whereafter the viable count remained constant to Station 9, that is close to 1 x 10^3cells.ml^{-1}.

The %P showed a similar trend, ranging between 0.01% - 0.04% for all supra-pycnocline stations inshore of Station 9. Between Station 9 and Station 10, however, there was a marked increase both in the viable count and the %P with %P, on the oceanic side of the front exceeding 1.0%. Samples taken below the pycnocline differed slightly from those above, showing a lower viable count up to Station 6, although the %P was slightly higher. These samples also showed an increase in %P from Station 6 to Station 10, unlike the supra-pycnocline samples where the increase occurred at the frontal station itself.

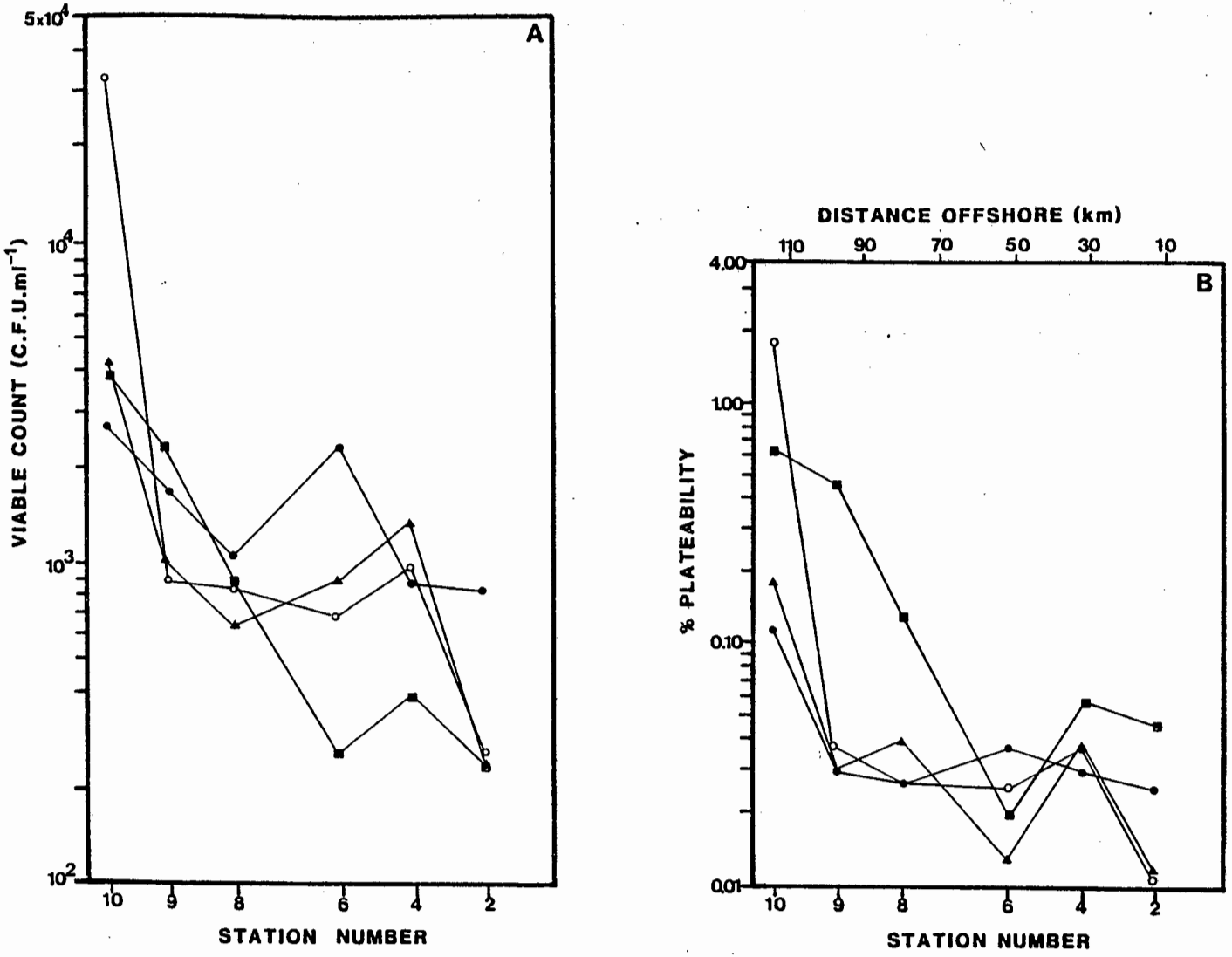

FIGURE 45: Changes in the viable count (CFU.ml^{-1}) and percentage plateability with distance from the shore, assessed at the 100% 1.1. (—●—) 25% 1.1. (—○—) on the pycnocline (—▲—) and below the pycnocline (—■—). (Fig. 45a: Viable count. Fig. 45b: Percentage plateability).

4.2.3.9 Generic distribution of plateable strains

On the basis of fermentation, motility, chromogenic, oxidase and catalase characteristics, plateable strains were assigned to one of five broad generic groupings at the three stations adjacent to or on the front (fig.47).

The plateable populations inshore of the front (Stations 8 and 9) were very similar to one another, and the 5 generic groups were represented in the following approximate proportions:

Pseudomonas	38 - 62%
Vibrio	10 - 38%
Enterobacteriaceae	0 - 37%
Acinetobacter	3 - 31%
Cytophaga/Flavobacterium	0 - 4%

At station 10, however, the plateable population was quite different, and at all depths tested, Pseudomonas strains dominated the population, the other 4 genera being poorly represented or entirely absent, as shown by the following ranges:

Pseudomonas	84 - 97%
Vibrio	0 - 8%
Enterobacteriaceae	0%
Acinetobacter	0 - 3%
Cytophaga/Flavobacterium	0 - 4%

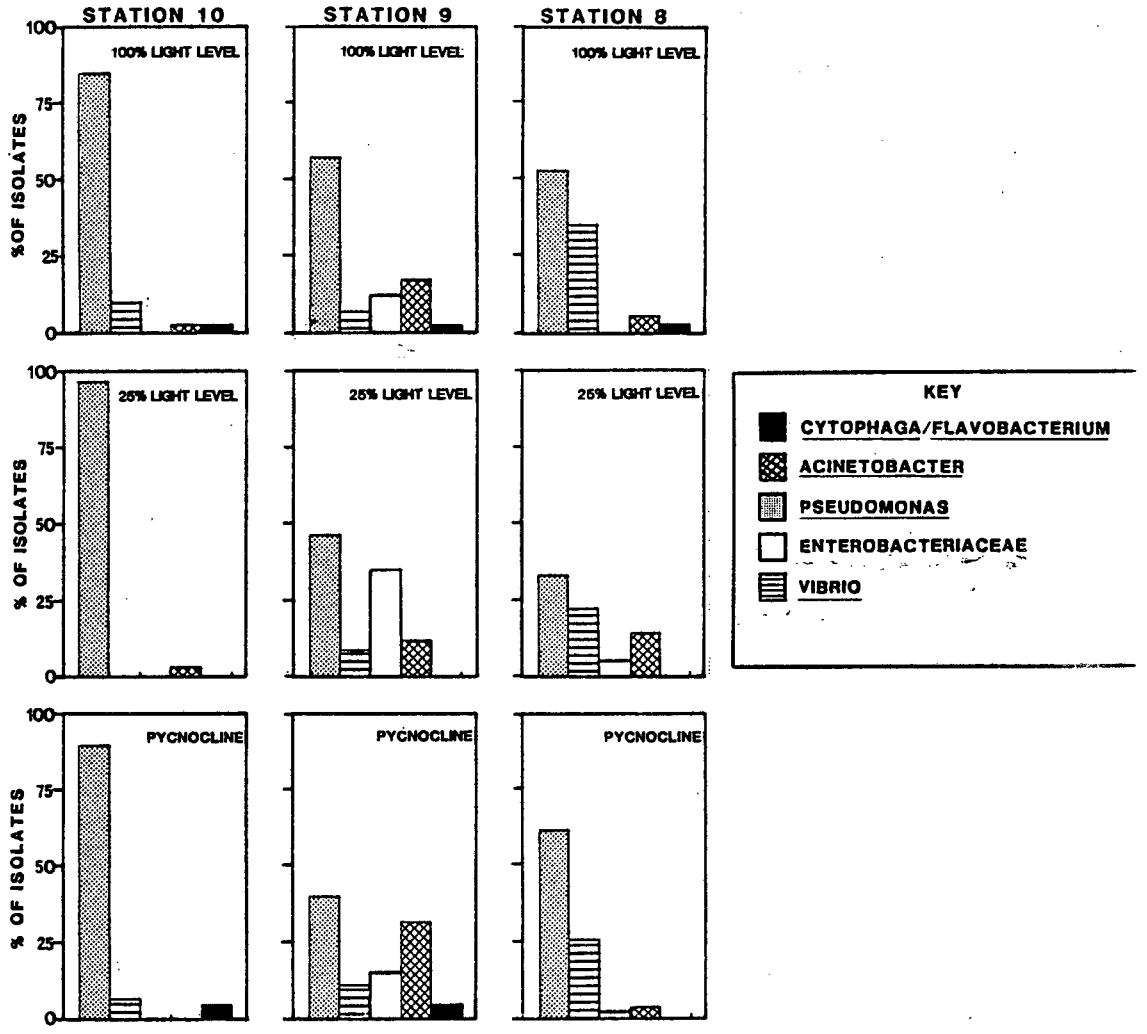

FIGURE 46: Distribution of bacterial genera in the plateable population at stations on, or adjacent to the front, at specified depths.

4.2.4 Discussion

The west coast of the Cape Peninsula is characterised by periodic upwellings of South Atlantic Central Water, which introduce nutrient rich waters to the surface (Andrews and Hutchings,1980). Such upwellings are produced by persistent south-easterly winds. During the period immediately prior to this survey, south-easterly winds blew for a period of 7 days (fig.34). Immediately prior to the survey a wind reversal occurred causing a relaxation of upwelling conditions. Nonetheless, coastal downwelling did not occur, and a body of recently upwelled waters was present (fig.36) identifiable by its low salinity (<34,8°/oo) and high NO_3 concentration (>10µg at NO_3-N. ℓ^{-1}), limited to the coastal stations (within 20km) by the shoreward movement of waters further offshore. This body of water had already been sunwarmed to 15°C so that no thermal upwelling front occurred. Within this column of recently upwelled water, an early phytoplankton bloom was detected. While Chl a concentrations here were higher than elsewhere on the transect (>4.0mg.m^{-3}) they were not as high as would be expected of a bloom in late log or stationary phase . Brown and Hutchings (1985) record Chl a levels of up to 250mg.m^{-3} at other times along the same U.M.L. In addition, the high nutrient levels suggest that little biological activity had yet taken place here (Barlow, 1982a, b).

A correlation was noted between Chl a levels (and hence, phytoplankton biomass) and bacterial biomass. This was not spatially invariable however, and appeared to depend on the physical and hydrographic characteristics of the water column as well as on the physiological state of the phytoplankton blooms. For example, in the recently upwelled coastal water, although Chl a concentrations were high, bacterial numbers and biomass were no higher than elsewhere in the surface waters and the plateability was low, suggesting that the bacterial population was in early log phase or still adapting from the nutrient starved conditions prevailing in the source waters. As discussed elsewhere (Section 3) bacterial numbers, biomass , plateability and activity tend to lag behind phytoplankton bloom development, and a similar effect has been noted by Lochte and Turley (1985).

Between 20 and 100km offshore the water column was characterised by the interaction of 2 major water bodies which could be distinguished on the basis of their salinity (fig.36). The first had a high salinity (>34.90 ^{o}/oo) and lay adjacent to the coastal upwelled water, and the second had a low salinity (<34.80 ^{o}/oo) and lay between 60 and 90km offshore. No distinction could be drawn between these bodies of water on the basis of nutrient chemistry or biological

characteristics and both are thought to have been aged upwelling plumes. Associated with them was an extensive phytoplankton bloom (fig.41) in which Chl a concentrations exceeded 4.0mg.m^{-3}. The nutrient concentrations however were very low, suggesting that this was a senescent patch. Further confirmation of this is provided by the species composition data of Hutchings et al. (1984) which show that the phytoplankton of this region was dominated by microflagellates characteristic of bloom senescence. Bacterial numbers and biomass were also high in this region (figs 42, 43) producing a distinct patch closely associated with the areas of high Chl a. Plateability was also low here, which would be expected in a senescent bloom (see Sections 2 and 3) where high D.O.C. levels no longer prevailed.

It was evident that while the phytoplankton distribution was limited to areas of upwelled water, associated bacterial populations extended beyond these patches to a large extent. The phytoplankton patch at the central stations terminated at about 88km offshore but the associated bacterial patch extended to 110km. In this region, a prominent and well defined front occurred, marking the convergence of oceanic water and the inshore waters of upwelled origin. This produced marked downwelling on the inshore face of the front, as evidenced

by the steep downturning of the nutrient isopleths (figs 38-40). The patch of high bacterial numbers and biomass was influenced by this downwelling and high bacterial numbers and biomass were evident as deep as 40 - 50m as a result (fig.42, 43). In addition there was evidence of accumulation of bacterial cells in this region, since distinct peaks are noted in the numbers and biomass present, relative to adjacent stations (figs 44). A slight increase in bacterial plateability in the surface water at Station 9 suggests that there may have been an increase in bacterial activity here but this was not marked. It must be noted, however, that there was no increase in the Chl a levels at the front, such as has been previously found (Hutchings et al, 1984, 1985).

On the oceanic side of the front nutrient concentrations were very low (figs 38-40), but a small patch of phytoplankton occurred in the subsurface waters (15 - 40m) (fig.41). While there was no increase in bacterial numbers or biomass associated with this patch, there was a marked increase in bacterial plateability, suggesting an increase in bacterial activity.

It was observed that the plateable bacterial population composition of the oceanic waters was quite different from that of the inshore waters adjacent to the front.

The oceanic population was composed mainly of obligately aerobic Pseudomonas strains, with very few Vibrio, Enterobacteriaceae, Acinetobacter or Cytophaga/Flavobacterium isolates, which are major components of the inshore populations (fig.46). It has previously been noted, both in the present study (Section 3) and elsewhere (Martin, 1980; Fukami et al., 1985 a, b) that a predominance of Pseudomonas strains is characteristic of bacterial bloom development. The results of Section 3 would suggest that this population had arisen in response to an increase in ambient nutrient resulting from the presence of the small phytoplankton bloom. There was, however, no concommitant increase in bacterial numbers or biomass. The oceanic bacterial population is possibly inherently different from that of the inshore stations both in terms of generic composition and response to nutrient availability. Thus, the increase in phytoplankton biomass and bacterial plateability at the front may have been the result of a response by a highly oligotrophically adapted population to the slight increase in nutrients across the frontal boundary, contrasted with the more eutrophic populations of the inshore region, which remained nutrient limited at the front.

In summary, it can be said that high bacterial numbers and biomass were limited to areas above the pycnocline

and were closely related to phytoplankton distribution, which in turn was determined both by physical hydrological characteristics and the nutrient chemistry of the water column. While centred around such areas of high phytoplankton density, the bacterial populations did extend beyond them and there was evidence of accumulation of bacterial biomass both at the pycnocline and at the front. The front also marked the convergence of two distinct bacterial populations, the one presumably highly adapted to oligotrophic conditions and the other more eutrophically adapted.

SECTION 4.3: THE RESPONSE OF BACTERIAL POPULATIONS TO CHANGES IN FRONTAL STRUCTURES OF THE BENGUELA UPWELLING SYSTEM IN THE REGION OF CAPE COLUMBINE (32°48'S, 17°49'E)

4.3.1 Introduction

Whereas previous surveys of the frontal zone of the Benguela system have provided short-term and seasonal information on the physical and biological features of the Benguella oceanic front, little information is available of changes associated with variability in the structure of the front itself. Surface features of the front in particular are extremely sensitive to changes in wind-stress and may vary within hours of a change in wind direction (Andrews and Hutchings, 1980; Lutjeharms, 1981; Hutchings et al., 1985; Taunton-Clark, 1985; Shannon, 1985b).

In the work described here, a transect perpendicular to the orientation of the oceanic front in the region of Cape Columbine (32°42'S,17°49'E) was consecutively sampled 4 times over a period of 5 days, to assess spatial (both with distance from the shore and with depth) and temporal changes in relation to changes in the frontal structure.

4.3.2 Materials and methods

4.3.2.1 Sampling procedure

All sampling was conducted from the R.S. Africana on a

cruise lasting from 6 - 13 December 1984. A transect was established, starting 5.56km offshore (Station 1) of Cape Columbine (32°48'S 17°49'E) with stations equidistantly placed at 5.56km (3 nautical mile) intervals to a total distance of 116.76km offshore (fig.1).

On the initial transect, a C.T.D. sampler was cast at each station, and a hydrographic profile was drawn which showed the presence of a front between stations 5 (27.8km) and 6 (33.36km). Accordingly, subsequent passages of the transect were limited to 55.6km offshore (Station 10).

The transect was sampled consecutively for a further 3 legs, using the same sampling stations on each leg. At each station, a C.T.D. cast was initially done to determine the hydrological features and water samples were taken at depths determined on the basis of the hydrological conditions in 30ℓ N.I.O. bottles fixed to a rosette sampler.

4.3.2.2 Nutrients and Chl a

NO_3, NO_2, SiO_3, PO_4 and Chl a concentrations were determined using the same methods described in Section 2.2.2

4.3.2.3 Bacterial numbers and biomass

Bacterial numbers and biomass were determined using the same methods described in Section 2.2.4.

4.3.2.4 Bacterial plateability

0.1ml aliquots of water from 0m, 10m, 20m, 50m, and 70 - 100m depth were plated in triplicate onto 0.1% Pep-SWA were appendix) on the fourth leg of the transect, and at each of the stations at which heterotrophic uptake activity was determined (see Section 4.3.2.5 below). In addition, plateabilities on 0.1% Pep-SWA were determined at various depths at Stn 3 (16.68km) on each of legs 2, 3 and 4. Viable counts and %P were determined following the methods described in Section 2.2.5.

4.3.2.5 Heterotrophic uptake activity

^{14}C universally labelled glucose and glutamic acid (Amersham) were used as radiotracers to determine the heterotrophic uptake activity. Glucose is commonly used as an experimental substrate (Meyer-Reil et al., 1979; Bolter, 1981; Gocke et al., 1977, 1983; Lochte, 1985) since it is the major dissolved free carbohydrate associated with phytoplankton (Liebezeit et al, 1980;

Ittekot et al. 1981), but dissolved amino acids are also important labile substances associated with phytoplankton blooms (Liebezeit et al., 1980) and are readily taken up by bacterioplankton (Dawson and Gocke, 1978). Uptake parameters (K^m, V^{max} and V^{max}/B) were determined at Stations 55.6km offshore (leg 4) and at 16.68km on legs 2 and 3, that is, in oceanic water and in inshore water before and during frontal development. In each case, uptake parameters were determined for populations 10m above the pycnocline and at least 15m below the pycnocline using methods described by Wright and Hobbie (1966), Wright (1978), and Hoppe (1978). 25ml samples were incubated in sterile glass containers maintained (shaking) at the temperature from which they originated.

These water samples were prefiltered through Whatman GF/C filters, and inoculated with the following final concentrations of substrate: glucose = 7nmol.l^{-1}, 50nmol.l^{-1}, 100nmol.l^{-1}, 200nmol.l^{-1}; glutamic acid = 9nmol.l^{-1}, 50nmol.l^{-1}, 100nmol.l^{-1}, 200nmol.l^{-1}. 2ml subsamples were taken at the time of inoculation, then at 0.5 and 1 hour, and thereafter at hourly intervals for a further 5 hours. The reaction was stopped by filtering through 0.22µm Gelman Nitrocellulose filters which were then rinsed with 0.1mmol.l^{-1} of the appropriate substrate in sterile

filtered seawater. Filters were dried on a warming plate and stored at -4°C until they were counted. For counting, filters were covered with 5ml of Instagel Scintillation fluid and counted in a Packard Tricarb Liquid Scintillation Counter.

Uptake rates were determined from linear regression estimations of the linear portions of uptake curves, and K^m and V^{max} statistics were determined using double reciprocal plots of V and S as described by Lineweaver et al. (1934). V^{max}/B (specific heterotrophic potential) was determined by reference to total biomass for the appropriate stations, according to the methods of Wright (1978), and Simon (1985).

In addition to determination of the uptake kinetic parameters, the rates of uptake of very low ($<10 nmol.l^{-1}$) substrate concentrations of glucose and glutamic acid were determined at stations 10 (leg 4), 7, (leg 4) 5 (leg 2) and 3 (leg 2 and 3). As before, uptakes by populations above and below the thermocline were determined for 6 hours using 7.0 $nmol.l^{-1}$ (final concentration) glucose and 9.0 $nmol.l^{-1}$ (final concentration) glutamic acid.

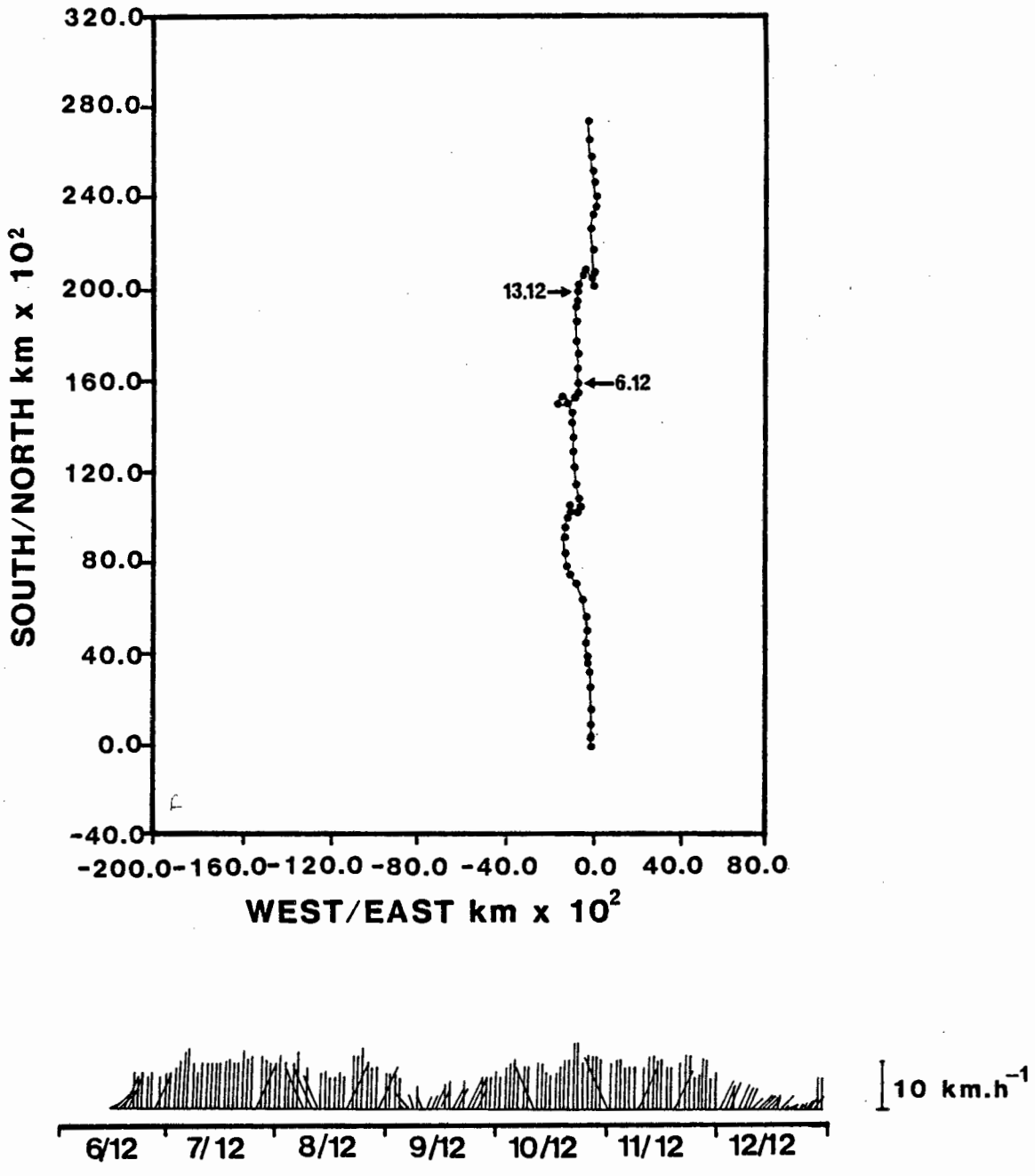

FIGURE 47: Wind stress vectors at Cape Columbine. (Fig. 47a: Progressive wind stress vector for the period 01/11/84 - 31/12/84. Fig. 47b: Wind stress vectors computed from shipboard data for the period of the cruise: 06/12/84 - 12/12/84).

4.3.3 Results

4.3.3.1 Wind

Wind stress data are presented as progressive wind stress vectors (fig.47a) for the period November - December 1984, and as daily vectors computed from shipboard data (fig.47b) for the duration of the cruise. These show that southerly winds ($<10 km.h^{-1}$) dominated the period prior to the cruise with only a mild reversal immediately before the survey started. Wind relaxations took place on 09/12/84 and 12/12/84. Prior to the start of the cruise winds were fairly strong and probably produced upswelling, but velocities were low ($<10 km.h^{-1}$) during the cruise.

4.3.3.2 Temperature

An initial transect (leg 1, fig.48a) showed no thermal evidence of upwelling. Water of 15°C was present at all stations inshore of Station 4 (22.24km), underlain by cooler waters, but with no distinct thermocline. Cold (<11.0°C) water occurred at a depth of 18m or more, even at the coastal station. Warm (>17°C) water of oceanic origin occurred at 30.58km offshore, creating a prominent thermal front between stations 4 and 7 with steep isotherms penetrating to depths of 25m - 30m, in

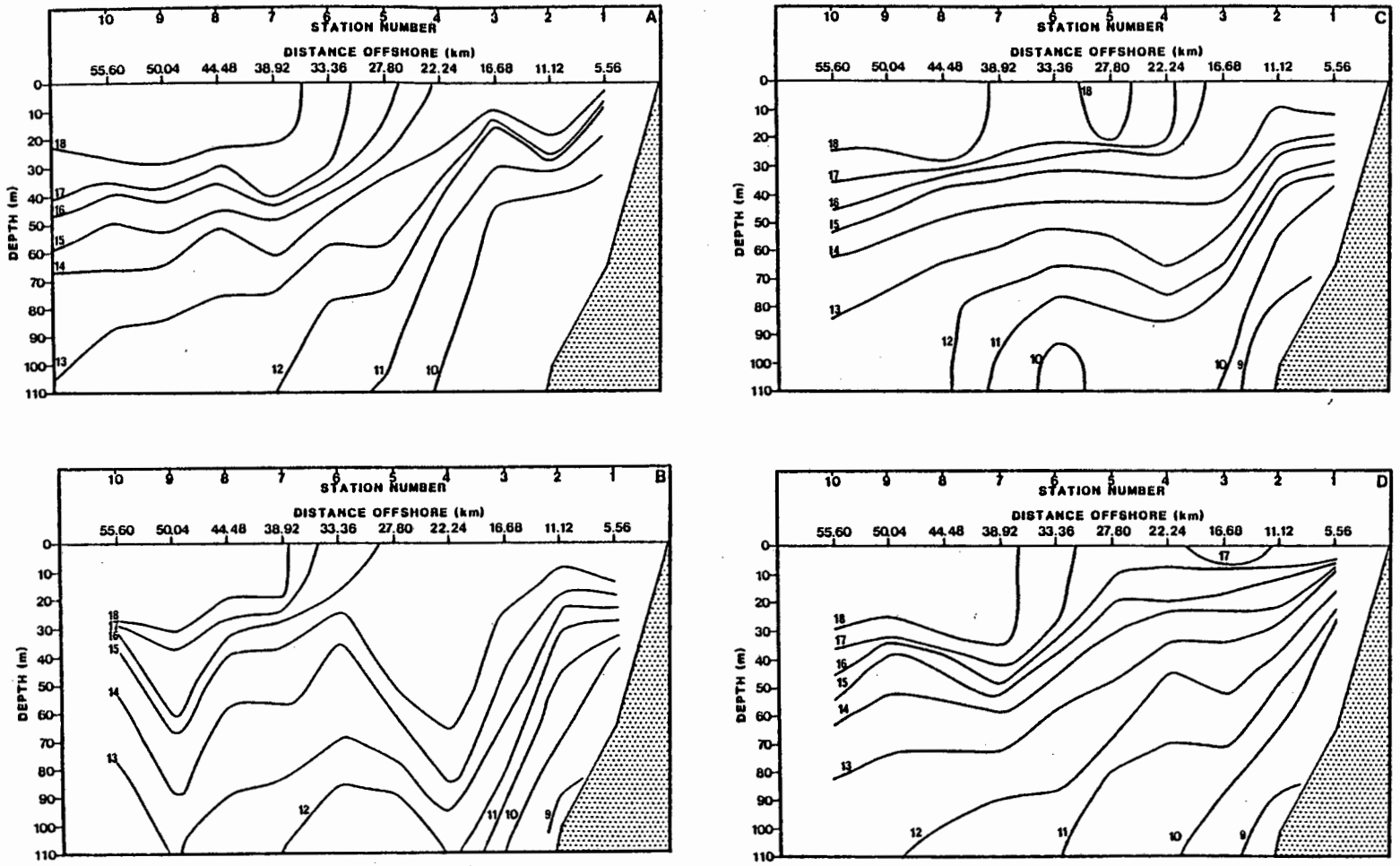

FIGURE 48: Profiles of temperature isotherms (^{o}C) for the Cape Columbine frontal transect. (Fig. 48a: leg 1. Fig. 48b: leg 2. Fig. 48c: leg 3. Fig. 48d: leg 4.)

which the surface temperature change was about 4°C in 13.50km. While stratification was weak on the inshore side of the front, it was more marked on the oceanic side with a deep thermocline at 40m - 50m depth.

On second passage (leg 2 - fig.48b) stratification was maintained at stations offshore of the front, but was less marked at the inshore stations. Sunwarming had caused the depression of the weak inshore thermocline to 60m depth at Stations 5 and 4 but the thermal front, though weakened, was maintained between station 5 and 7.

On third passage, a large body of warm (>18°C) water of oceanic origin had been introduced into the central waters between 25.58km and 32.00km offshore. This resulted in the formation of a new front lying between 18.00km and 25.00km offshore, with vertical isotherms extending to 25m depth. The oceanic water (temperature >7°C) previously noted was maintained in much the same position as before however (fig.48c).

On fourth passage, the patch of warm oceanic water had moved out of the central waters, and a weak front (2°C in 6km) had again formed between 30.00km and 37.00km offshore. The thermal structure of the front had been

considerably weakened by extensive sunwarming of the waters inshore of it, but vertical isotherms at the front extended to 35m depth. Sunwarming of the waters inshore of the front had resulted in marked stratification although no prominent thermocline was present (fig.48d).

4.3.3.3 Salinity

On first passage, a large body of low salinity (<35.0^{o}/oo) water was present at stations inshore of 25.00km (fig.49a), extending from below 130m depth to the surface in a manner typical of that of upwelled water. Offshore of Station 6 lay a body of oceanic water of salinity >35.4^{o}/oo: as a result, a prominent salinity front was present, with vertical isohalines extending between 45m to 130m depth, between stations 4 and 6. Offshore of this front, isohalines were horizontal, producing a weak halocline between 35m and 65m depth.

During the second passage (leg 2 - fig.49b) the coastal body of upwelled water, still bounded by a prominent salinity front, was somewhat reduced in extent, now lying closer than 20.00km inshore. This was evidently effected by the shoreward movement of the central waters which also undercut the upwelled water at 10m - 20m depth, producing a shallow tongue water of low salinity waters between

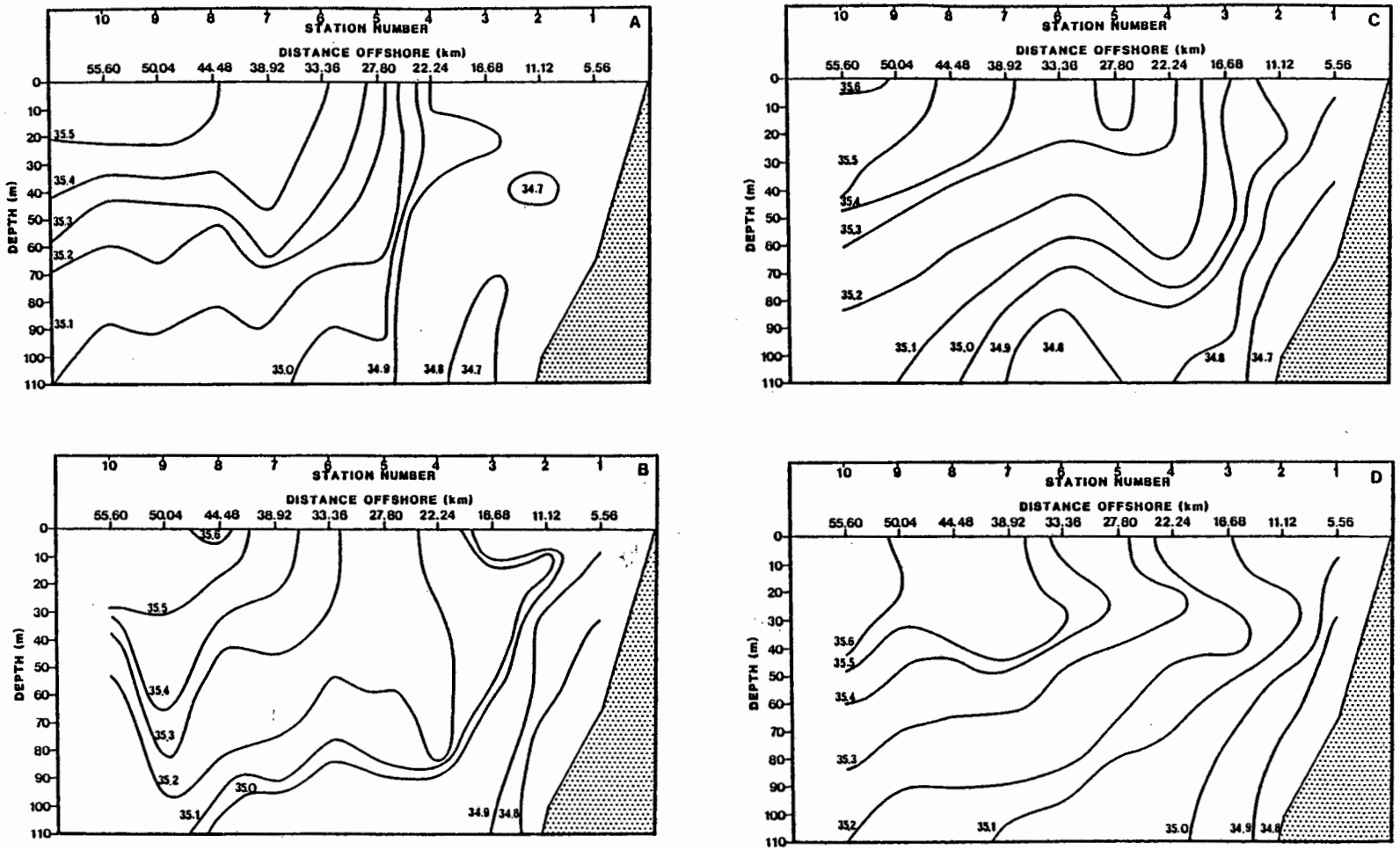

FIGURE 49: Profiles of salinity isohalines (^{o}/oo) for the Cape Columbine frontal transect. (Fig. 49a: leg 1. Fig. 49b: leg 2. Fig. 49c: leg 3. Fig. 49d: leg 4.)

11.00km and 20.00km offshore. A second salinity front, coinciding with the thermal front, lay between 32.00km and 40.00km offshore, marking the convergence of the oceanic waters with the central water. It is suggested that this separation of the salinity fronts and the compression of the inshore body of upwelled water was the result of the approach of the patch of oceanic water visible during the third passage (leg 3 - fig.49c). With the subsequent movement of this body of water out of the area, augmented by relaxation of the onshore winds, the surface waters above 40m flooded back offshore, as shown by the flexure of the vertical isohalines on leg 4 (fig. 49d). At completion of the survey, no prominent salinity front was present, and only a weak halocline was present from 33.00km offshore at a depth of 30m to 60m.

4.3.3.4 Density (σ^t)

Since the σ^t value is dependant upon both salinity and temperature, it reflects the interaction of these two parameters. Areas with closely spaced isopycnels are assumed to be relatively more stable than those without.

Despite the fact that prominent thermoclines and haloclines were not a feature of any of the transects, a profile of σ^t values for the initial transect (leg 1 -

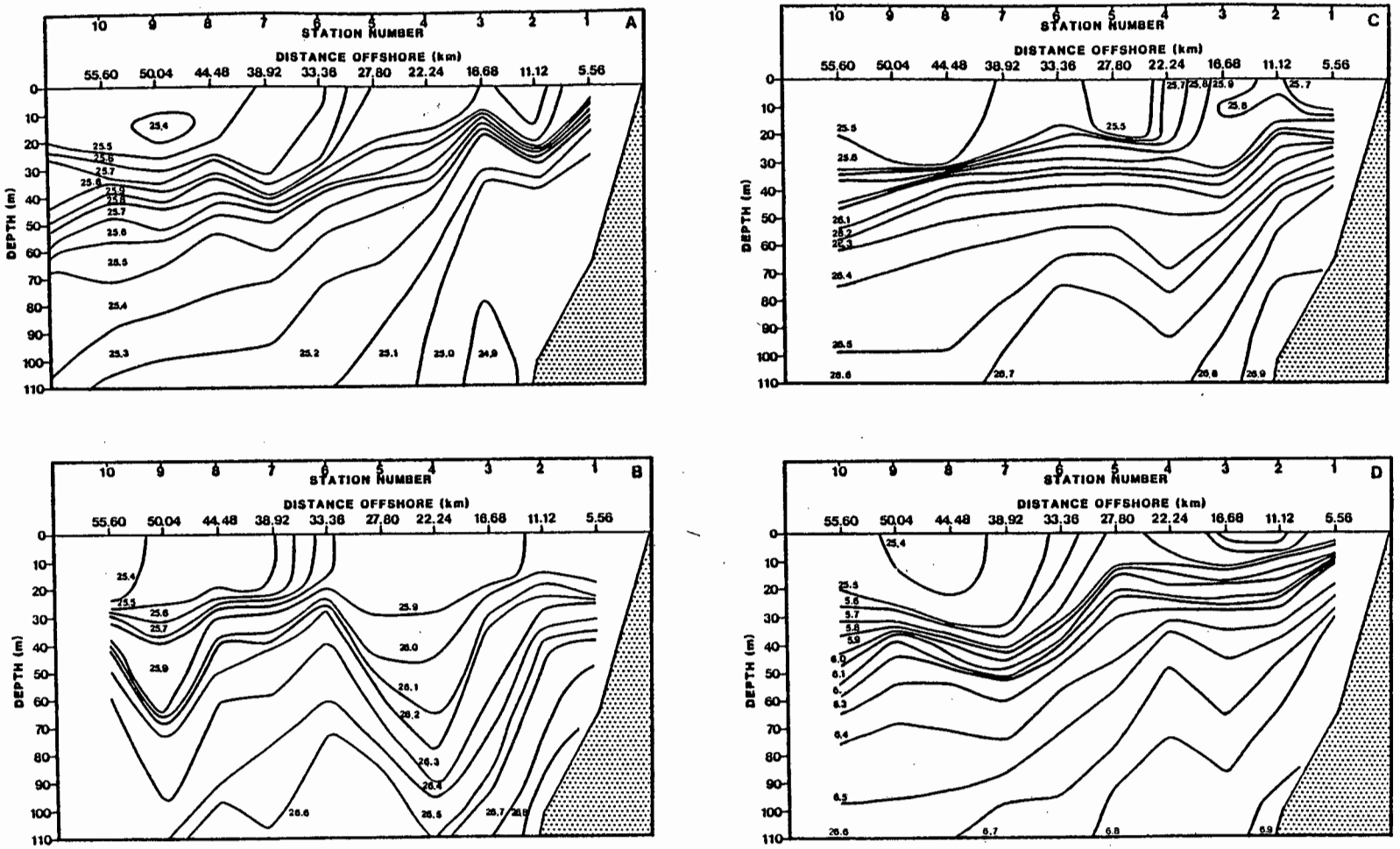

FIGURE 50: Profiles of density (σ^t) isopycnels for the Cape Columbine frontal transect. (Fig. 50a: leg 1. Fig. 50b: leg 2. Fig. 50c: leg 3. Fig 50d: leg 4.)

fig.50a) showed a prominent pycnocline inshore of 16km, at a depth of 6m to 30m. Further offshore, it was discernable but considerably weaker. The thermal and salinity fronts previously discussed (figs 48, 49) resulted in the production of vertical isopycnels in a fairly prominent offshore front (27.80km - 33.36km) at the convergence of the oceanic water (σ^t< 25.7) with the central waters (σ^t> 25.9). The convergence of upwelled coastal water (σ^t = 26.1) with these central waters resulted in a complex and weak secondary front between 6.00km and 16.00km offshore. The second transect (leg 2 - fig.50b) showed a strengthening of the σ^t front 33.00km - 39.00km offshore with vertical isopycnels to 20m depth. The 2° upwelling front was maintained but considerably weakened as evidenced by the complex isopycnel distribution inshore of it, which suggested extensive mixing above the pycnocline inshore of 16km. With the introduction into the central waters of low density (σ^t<25.6) oceanic waters and development of a thermal front coincident with the salinity front between 17.79km and 23.35km offshore, a prominent and deep (25m) σ^t front formed in this region (fig.50c). Below it, a well developed pycnocline was established at 15m to 30m depth. Inshore of this front and above the pycnocline, mixing appeared extensive, probably as a result of the frontal movement and sunwarming of the waters.

With the movement out of the central waters of the oceanic water mass (fig.50d) the front reformed between 29.00km and 35.00km offshore. Extensive sunwarming of the waters inshore of this led to the development of dense stratification above a pycnocline ranging in depth from 5m to 15m. Offshore of the front, which extended to 40m depth, the pycnocline lay much deeper, between 20m and 35m.

4.3.3.5 Nitrate

Profiles of NO_3 distribution are shown in figs 51a-c. Throughout the survey there was a strong association between the distribution of NO_3 through the water column and the density (σ^t) structure. The major source of NO_3 to the surface waters arises through upwelling of NO_3 rich South Atlantic Central Water. The persistance of highly stratified conditions tends to lead to impoverishment of the surface waters as nutrients are utilised by biological activity. There was a marked increase in NO_3 concentrations with depth. Low surface values (<1.0µg at NO_3-N.l^{-1}) were initially noted at surface stations inshore of 21.00km, but in the waters above the pycnocline at the central stations (25.00km - 35.00km), NO_3 values were higher (>2.0µg at NO_3-N.l^{-1}). The position of the σ^t front (27.80km - 33.36km) marked a sudden drop

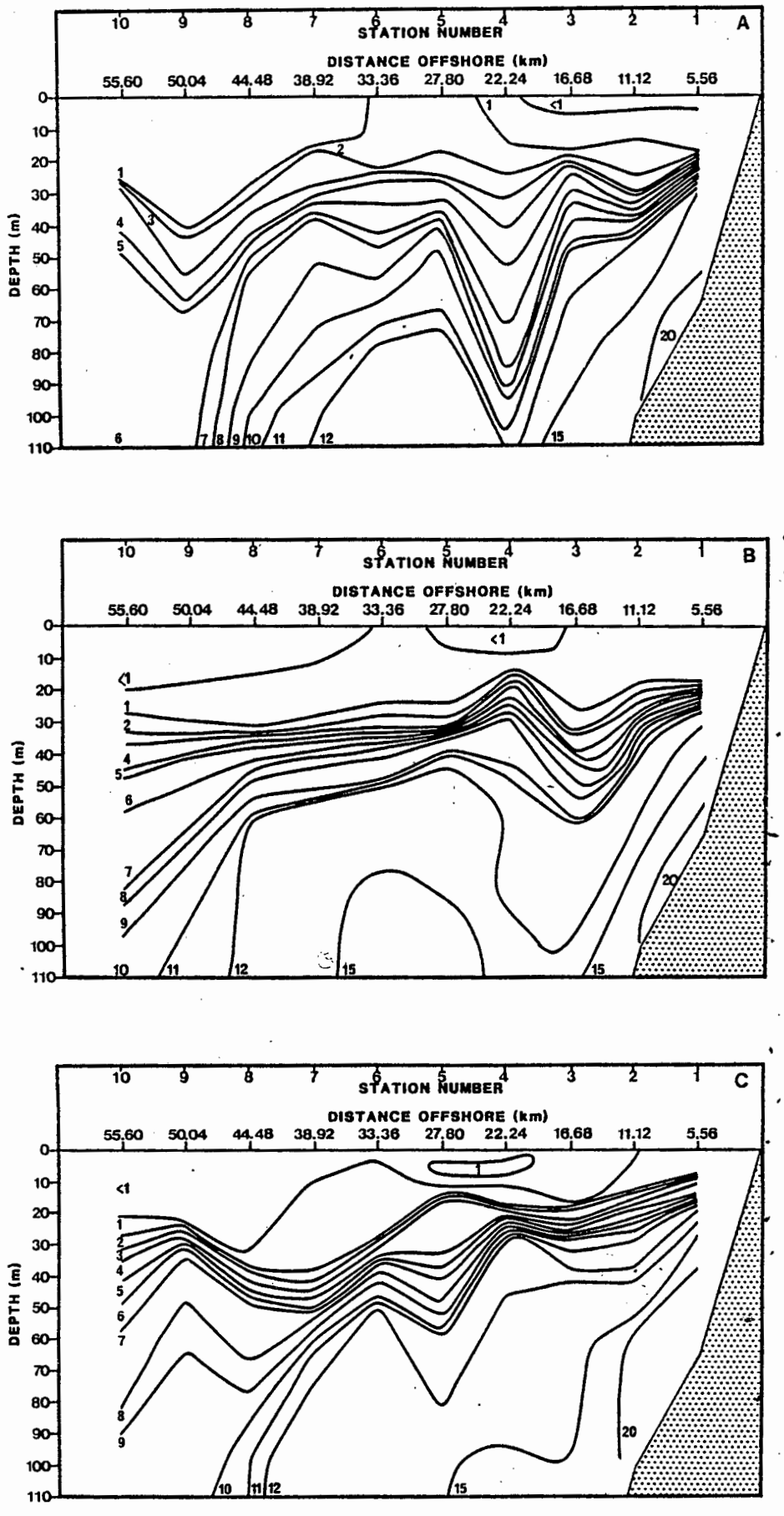

FIGURE 51: Profiles of NO_3-N isopleths (µg at NO_3-N.l^{-1}) for the Cape Columbine frontal transect. (Fig. 51a: leg 2. Fig. 51 b: leg 3. Fig. 51c: leg 4.)

in the NO_3 values although the oceanic concentrations were above limiting levels (>1.0µg at NO_3-N.l^{-1}) (fig.51a).

With the movement of a body of oceanic water into the central surface water stations, surface NO_3 levels were observed to fall below <1.0µg at NO_3-N.l^{-1} (fig.51b), but the resultant formation of a front between 17.78km and 23.35km caused extensive mixing of the inshore waters to a depth of 30m (fig.51b), with a resultant increase in the NO_3 levels to >1.0µg at NO_3-N.l^{-1}, particularly in the surface waters. In the offshore waters, the NO_3 levels were observed to fall below 1.0µg at NO_3-N.l^{-1} to depths of 20m. They remained below limiting levels for the duration of the survey.

With the formation of the front between 29.00km and 35.00km offshore (fig.50d) extensive mixing of NO_3 rich and poor water was observed to occur adjacent to it (fig.51c) particularly between 22km and 28km offshore, to a depth of 35m.

In summary, it was observed that NO_3 concentrations in waters above the pycnocline were low, probably due to biological activity, but that even in the absence of active upwelling, the presence of a marked front led

to some vertical mixing, which appeared to maintain localised NO_3 concentrations above limiting levels.

4.3.3.6 Nitrite

Profiles of nitrite distribution are presented in figures 52a - c. Surface distributions of NO_2 were very similar to those of NO_3 (figs 51a - c) and in general were very low above the pycnocline (<0.1µg at NO_2-N.l^{-1}). There was a marked increase in NO_2 concentration with depth, and a prominent NO_2 maximum, probably arising due to bacterial respiration of NO_3 by NO_3 reduction, was perennially present between 35m and 100m depth. Within this NO_2 maximum, NO_2 concentrations were up to twice those of the surrounding waters. Above 15m, NO_2 levels remained below 0.1µg at NO_2-N.l^{-1} at all stations greater than that 39km offshore, but inshore of this, fluctuations in NO_2 levels above the pycnocline were observed to occur, and the presence and changing position of the front resulted in mixing and elevation of NO_2 levels above 0.1µg at NO_2-N.l^{-1} in a similar manner to that described for NO_3.

4.3.3.7 Phosphate

Profiles of PO_4 concentrations in the water column are

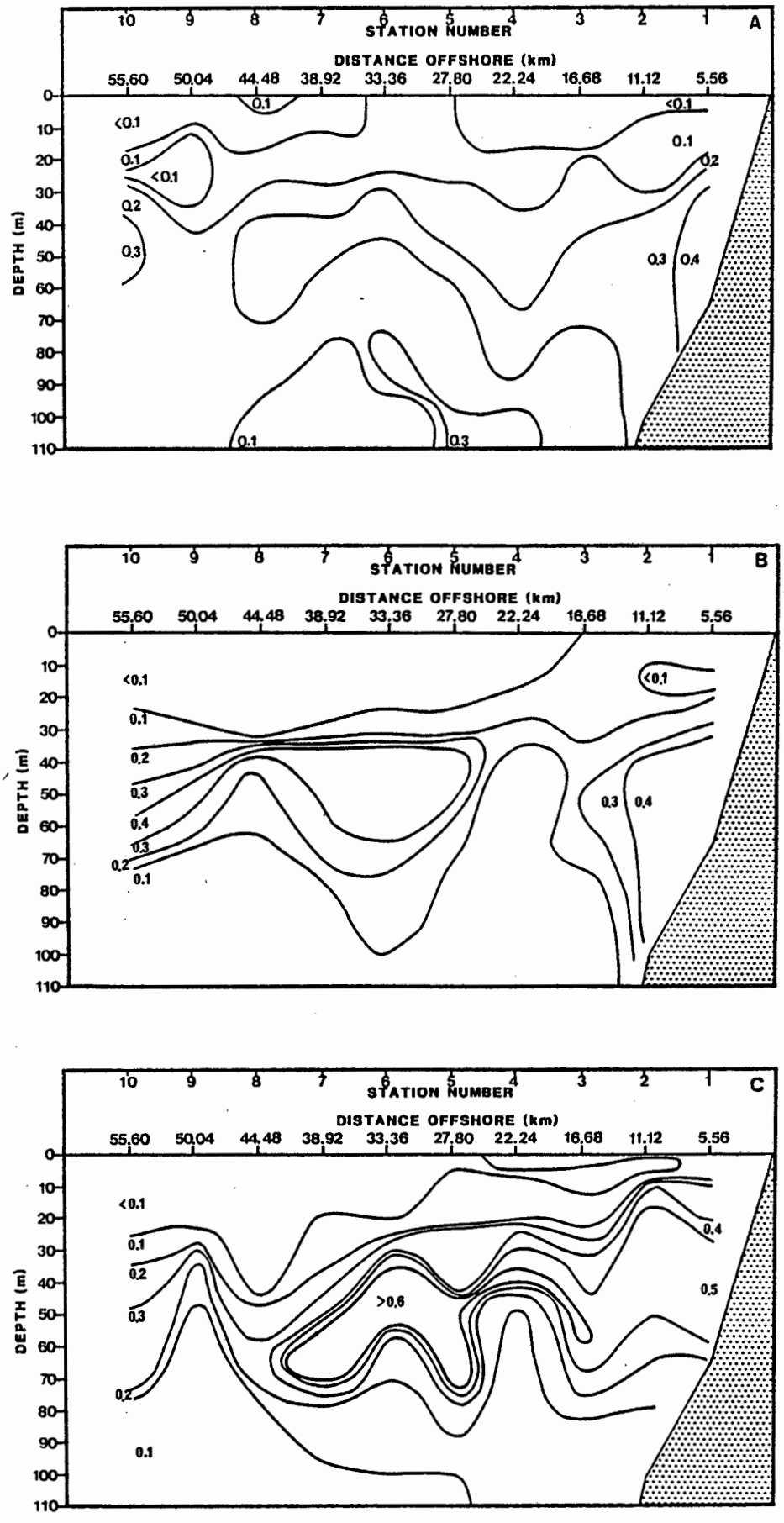

FIGURE 52: Profiles of NO_2 isopleths (µg at NO_2-N.l^{-1}) for the Cape Columbine frontal transect. (Fig. 52a: leg 2. Fig. 52b: leg 3. Fig. 52c: leg 4.)

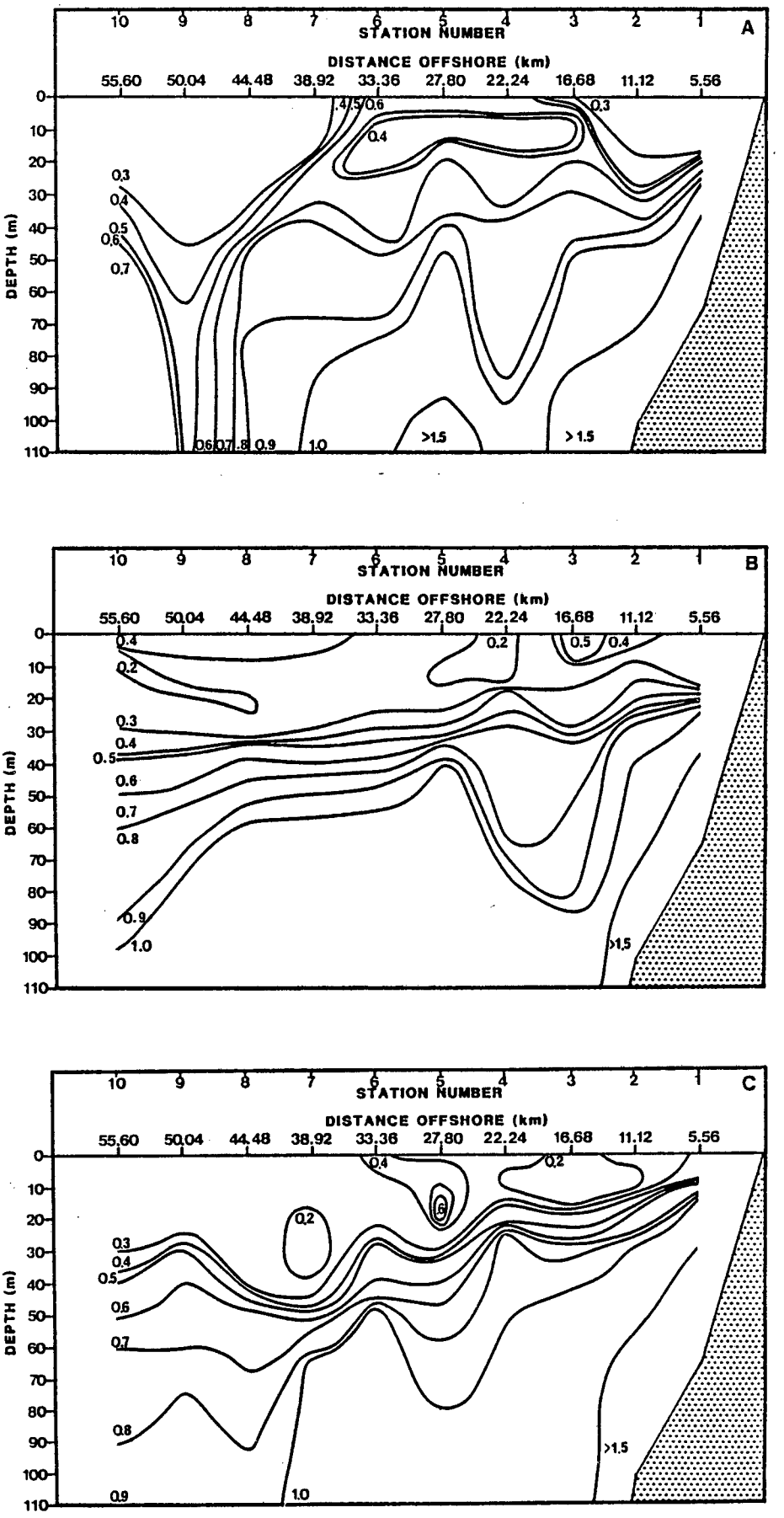

FIGURE 53: Profiles of PO_4 isopleths (µg at.l^{-1}) for the Cape Columbine frontal transect. (Fig. 53a: leg 2. Fig. 53b: leg 3. Fig. 53c: leg 4).

presented in figs 53a-c. There was again a close correlation between the PO_4 distribution and the density structure, and highest PO_4 concentrations were recorded in the South Atlantic Central Water, with maximum values above the bottom sediments. Initially (leg 2 - fig.53a) a clear nutrient front coincided with the position of the σ^t front, with vertical nutriclines marking the boundary between low PO_4 offshore waters ($<0.4\mu g\ at.l^{-1}$) and PO_4 rich central water ($>0.6\mu g\ at.l^{-1}$), with nutrient rich waters in this region extending to 40m depth. Inshore of 20km and closely associated with the salinity front which was produced by the recently upwelled water, the PO_4 concentrations were low ($<0.5\mu g\ at.l^{-1}$), probably as a result of biological activity in this area, and again a nutrient front arose coinciding with the position of the secondary upwelling front.

With the introduction into the central waters of low nutrient oceanic water (fig.53b) the PO_4 concentration of the central waters above the pycnocline dropped, but inshore of the newly formed front, the concentration of PO_4 was maintained, falling rapidly once the front had reformed further offshore and stratification again marked the inshore waters. With the formation of the front 29.00km to 35.00km offshore, a localised patch with

elevated PO_4 levels was again observed at the inshore face of the front (fig.53c) strongly suggestive of deep mixing at the frontal face.

4.3.3.8 Particle distribution

Particle distribution profiles are shown in figs 54a-c. In general particle concentrations decreased with depth and increasing distance from the shore, but the distribution was patchy. Initially (leg 2 - fig.54a) a large, discrete patch (5.0 x $10^6 \mu m^3.ml^{-1}$), extending to 20m depth was associated with the 2° upwelling front in this region. This patch was maintained through leg 3 (fig.54b) being compressed and deepened by the advent of the front at Station 3. Behind the front, the interposition of oceanic water had a low total particle volume (2.0 x $10^6 \mu m.^3 ml^{-1}$). With the disappearance of this tongue of oceanic water and the movement of the front further offshore, the particle patch was broken up and distributed through the deeper waters of the central stations (fig. 54c). A second patch was observed to develop at the coastal station to a depth of 20m during the latter part of the survey.

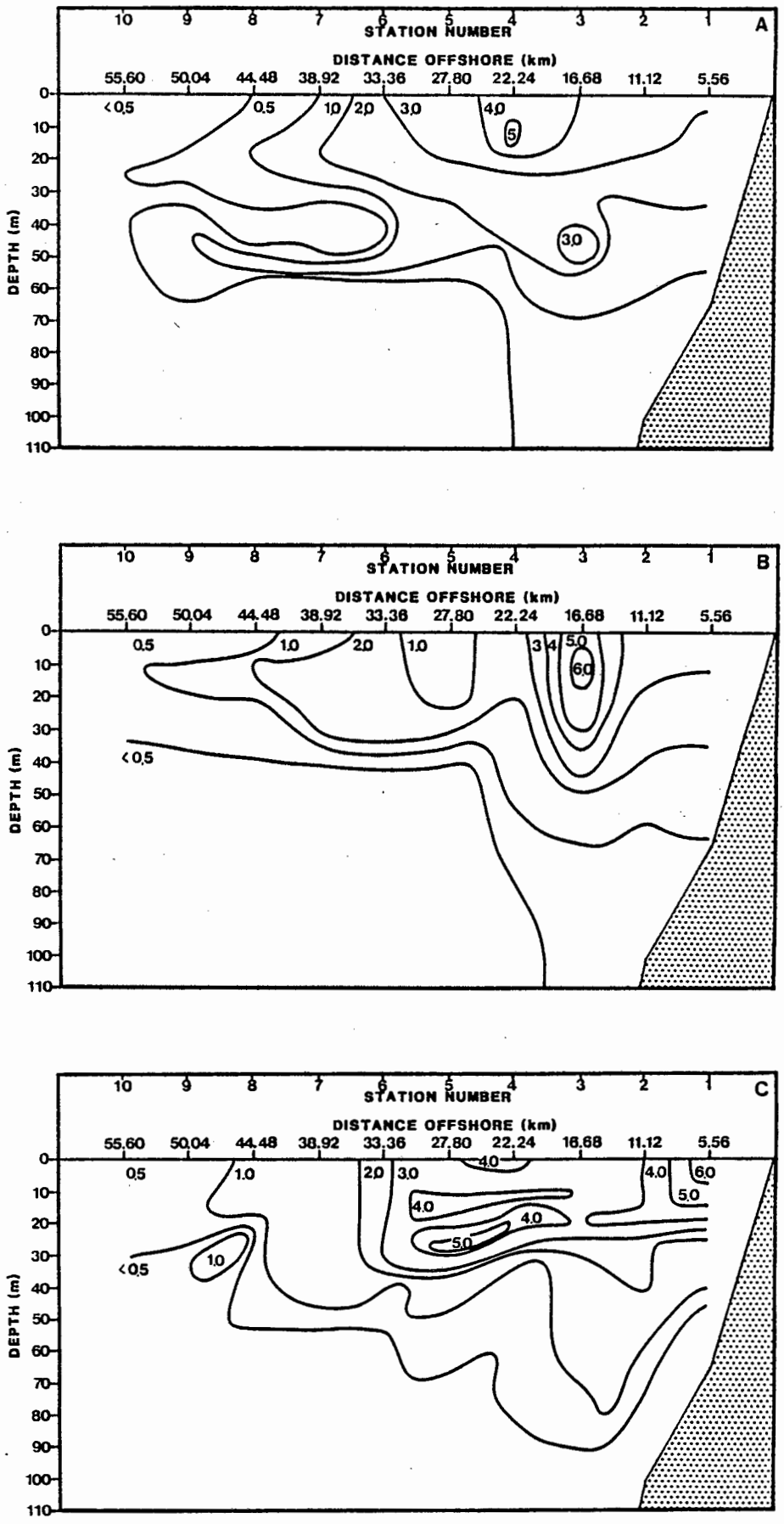

FIGURE 54: Profiles of the distribution of P.O.M. ($\mu m^3 . m^{-3}$) for the Cape Columbine frontal transect. (Fig. 54a: leg 2. Fig. 54b: leg 3. Fig. 54c: leg 4).)

4.3.3.9 Chlorophyll a

Chl a distribution profiles are shown in figs 55a-c, and concentrations at specific depths along the transect lines are shown in figs.58 - 60.

The distribution of Chl a remained relatively stable throughout the survey. Concentrations above $1mg.m^{-3}$ were limited to waters of upwelled origin, while oceanic waters beyond 38.92km offshore had Chl a concentrations less than $0.5mg.m^{-3}$. The underlying South Atlantic Central Water showed concentrations of less than $0.001mg.m^{-3}$.

Initially (leg 2 - figs 55a, 58) the position of the thermal front marked the outer boundary of the $>1.0mg.m^{-3}$ Chl a distribution 37.00km offshore. At this point highest Chl a values were recorded inshore of 16.70km, with a patch of Chl a $>5.0mg.m^{-3}$ at the innermost station, extending to 30m depth and out to Station 3 (16.68km) at 15m depth, forming a subsurface Chl a maximum. The distribution of this patch was closely correlated with the distribution of the recently upwelled water identified at the inshore stations, but as the nutrient levels above the pycnocline were low, and those in the surface waters very low (figs 51 - 53), this was judged to be an aged bloom. A second bloom was identified

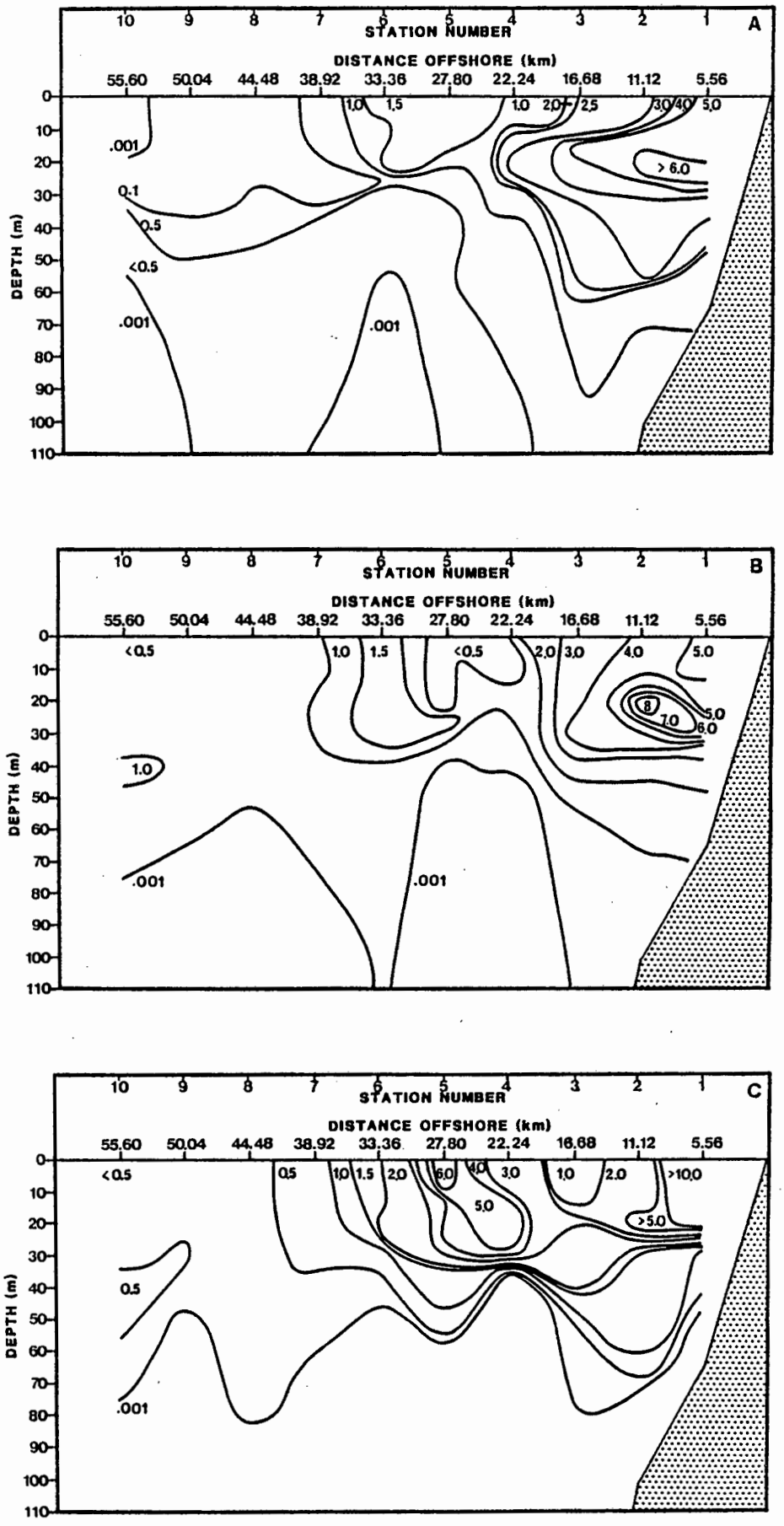

FIGURE 55: Profiles of the distribution of chlorophyll a ($mg.m^{-3}$) for the Cape Columbine frontal transect. (Fig. 55a: leg 2. Fig. 55b: leg 3. Fig. 55c: leg 4).

in the central waters lying between 23.00km and 35.00km offshore and extending from the surface to 25m depth.

The introduction into the central waters of low nutrient, low Chl a oceanic water resulted in the disruption of the offshore phytoplankton bloom and a drop in Chl a concentrations at the central stations (fig.55b), with the bloom being compressed and deepened at Station 6 to a depth of 35m. The formation of a front in the region of Station 3 resulted in mixing of the subsurface Chl a maximum with surface waters and with deeper waters, raising the Chl a concentration in the region from the surface to 45m depth. As a result a clear Chl a front arose distinguishing the inshore waters (Chl a $>3.0mg.m^{-3}$) from the central waters in which the thermal and ^{t} fronts lay (Chl a $<1.5mg.m^{-3}$).

With the passage of the tongue of oceanic water from the central waters and the re-formation of the front between 29.00km and 35.00km offshore, extensive mixing followed by sunwarmed stratification took place at the inshore stations. As a result, a high Chl a concentration was observed to arise at stations inshore of 10.00km to a depth of 22m, in which the Chl a concentration exceeded $10mg.m^{-3}$, rising as high as $25.19mg.m^{-3}$.

The offshore bloom previously noted at Station 6 (33.33km) now covered a large area, extending from the surface at Stations 6 and 5 (where the surface Chl a value was 6.18mg.m^{-3}) to 30m depth at Station 4 (5.93mg.m^{-3}), while all stations inshore of 33km showed increased Chl a values above the pycnocline.

The development of these blooms is also clearly demonstrated in figs 58 - 60, showing the horizontal distribution of Chl a at various depths.

4.3.3.10 Numbers and biomass of bacteria

Profiles of the numerical distribution of bacteria (cells x 10^6.ml^{-1}) are presented in figs 56a-c and those of bacterial biomass (µg.bacterial C.l^{-1}) in figs 57a-c. Horizontal distributions at specific depths are shown in figs 58 - 60, and selected vertical distribitions in relation to vertical physical and nutrient factors are shown in figs 61a-c.

Throughout the survey numerical and biomass distributions were similar. In general there was a decrease in both numbers and biomass with depth, and the pycnocline marked a disjuncture in the vertical distribution, the upper pycnocline boundary

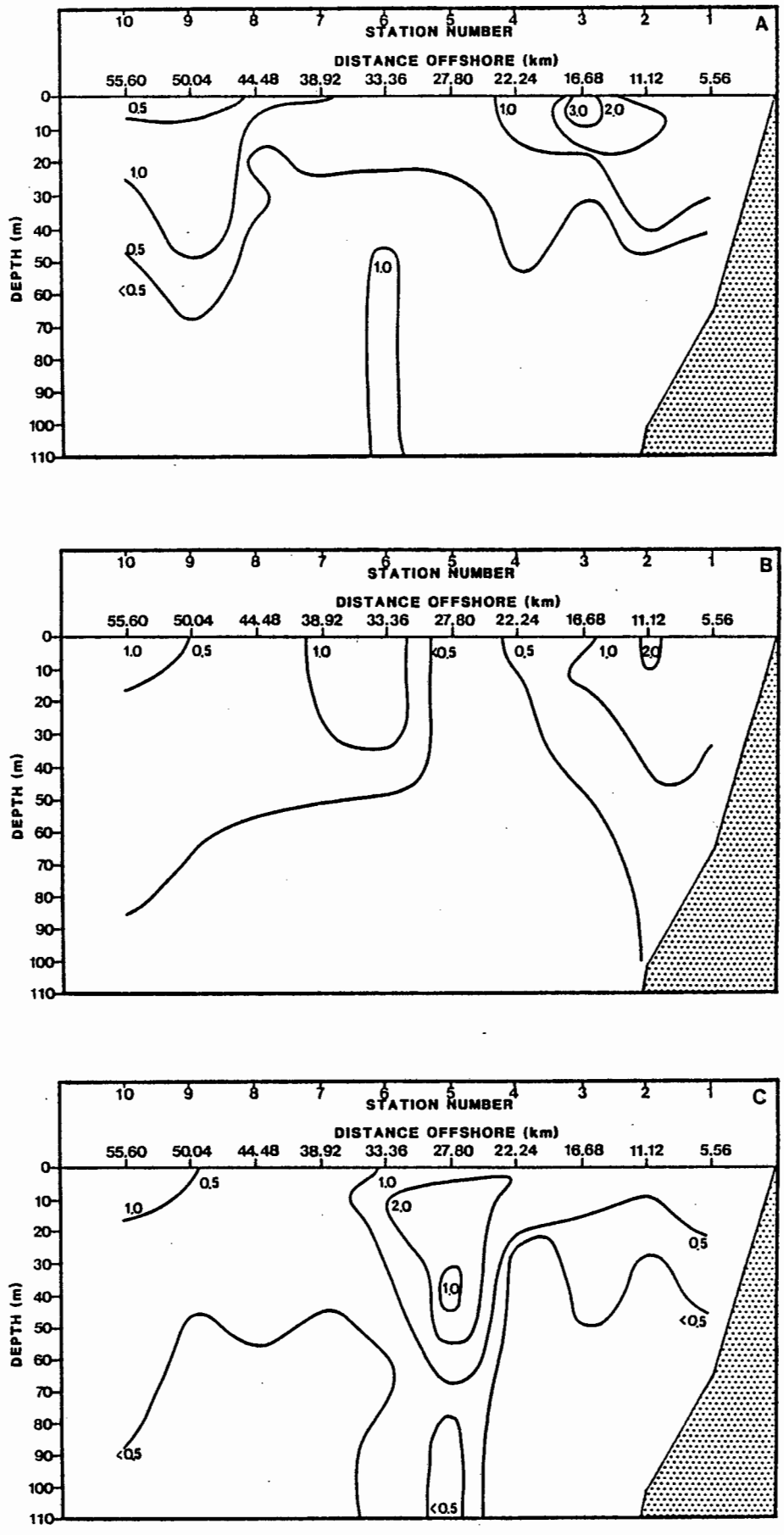

FIGURE 56: Profiles of the numerical distribution of bacteria (cells x 10^6.ml^{-1}) for the Cape Columbine frontal transect. (Fig. 56a: leg 2. Fig 56b: leg 3. Fig. 56c: leg 4).

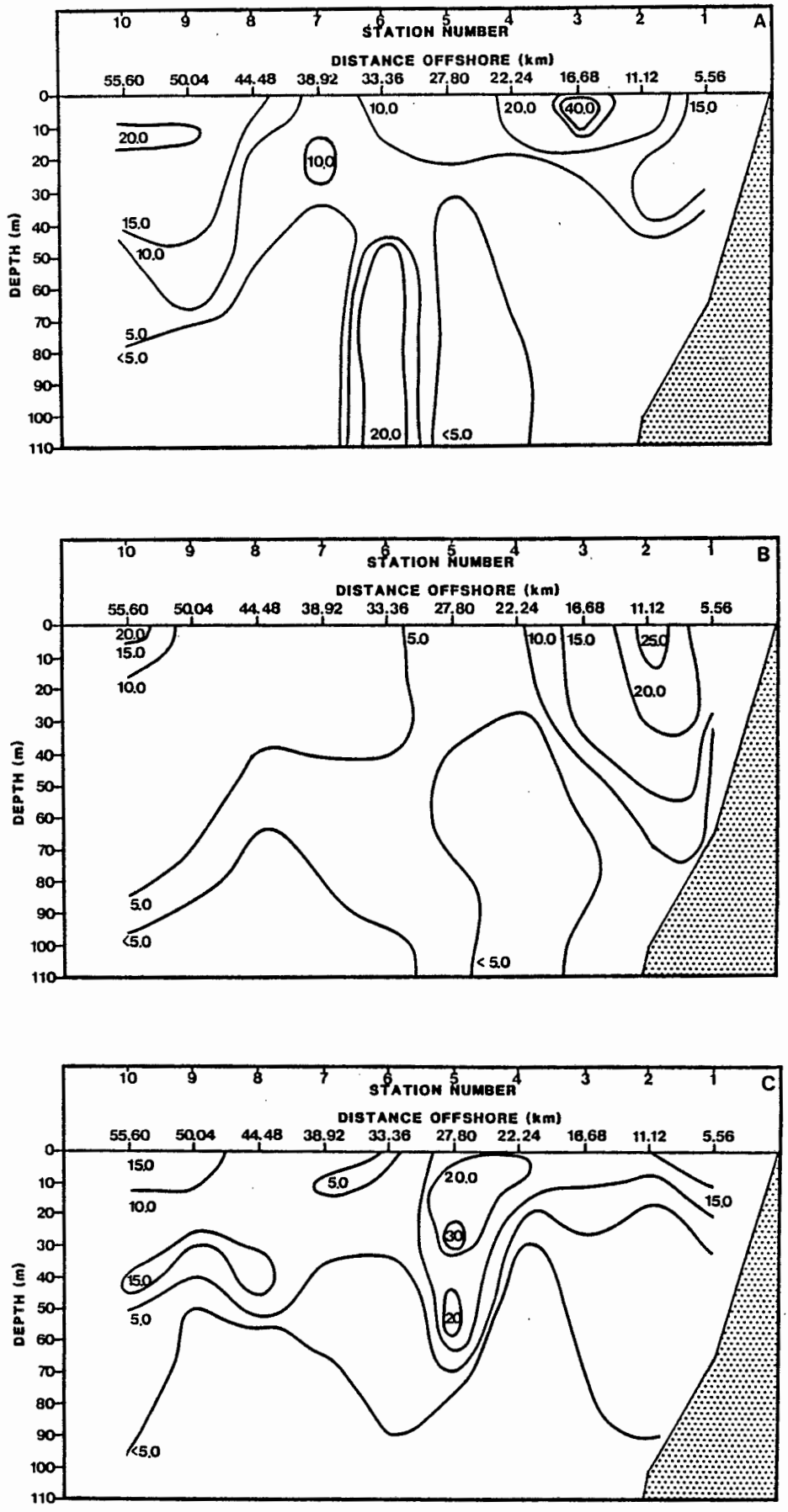

FIGURE 57: Profiles of the distribution of bacterial biomass ($\mu g\ C.l^{-1}$). (Fig. 57a: leg 2. Fig. 57b: leg 3. Fig. 57c: leg 4).

marking the 0.5 x $10^6.ml^{-1}$ distribution limit, particularly at the start of the survey (figs 56, 61).

The distribution of both numbers and biomass above the pycnocline was unstable and patches were noted which rapidly changed position and size along the transect. The inshore face of the front was observed to mark the biomass >10.0µg $C.l^{-1}$ boundary so that, in the areas inshore of the front, movement of the front resulted in rapid compression and expansion of the biomass distribution. The area of the front itself was observed to have low population densities (numbers = 1.00 x $10^6.ml^{-1}$, biomass = 10.0µg.$C.l^{-1}$) but densities in both the far offshore and inshore areas were similar, leading to a bimodal horizontal distribution (figs 58, 59, 60).

High numbers (3.75 x $10^6.ml^{-1}$) and biomass (30.0µg $C.l^{-1}$) of bacteria were initially recorded at 16.68km offshore, forming a distinct patch corresponding to the distribution of recently upwelled water and the phytoplankton bloom already described (figs 55a, 58). The overall bacterial distribution inshore of the front bore a strong similarity to the distribution of Chl a (fig.58). Numbers and biomass of bacteria in the region of the front were low, but those of the oceanic waters were similar to those of the inshore stations.

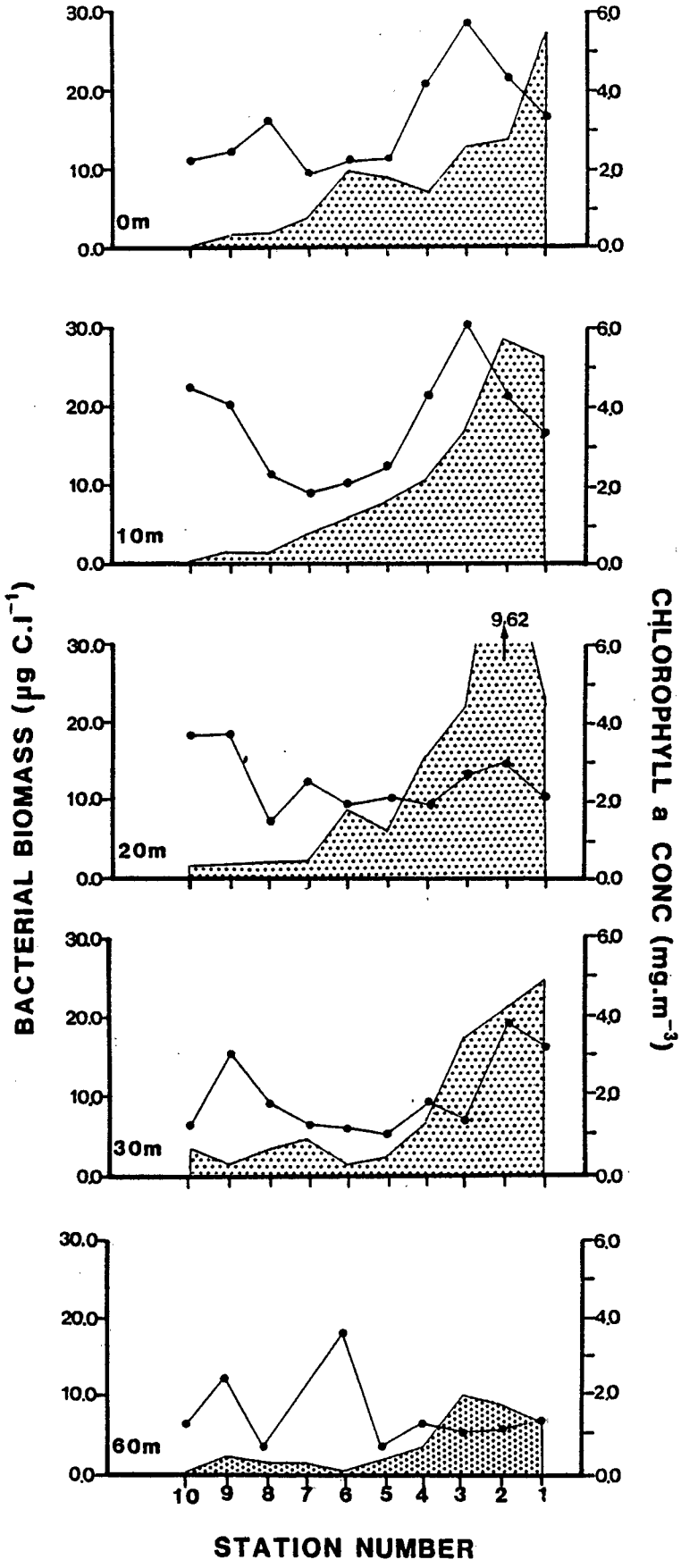

FIGURE 58: Horizontal distribution of bacterial biomass (—●— : µg $C.l^{-1}$) and chlorophyll a (▩ : $mg.m^{-3}$) at specified depths: leg 2.

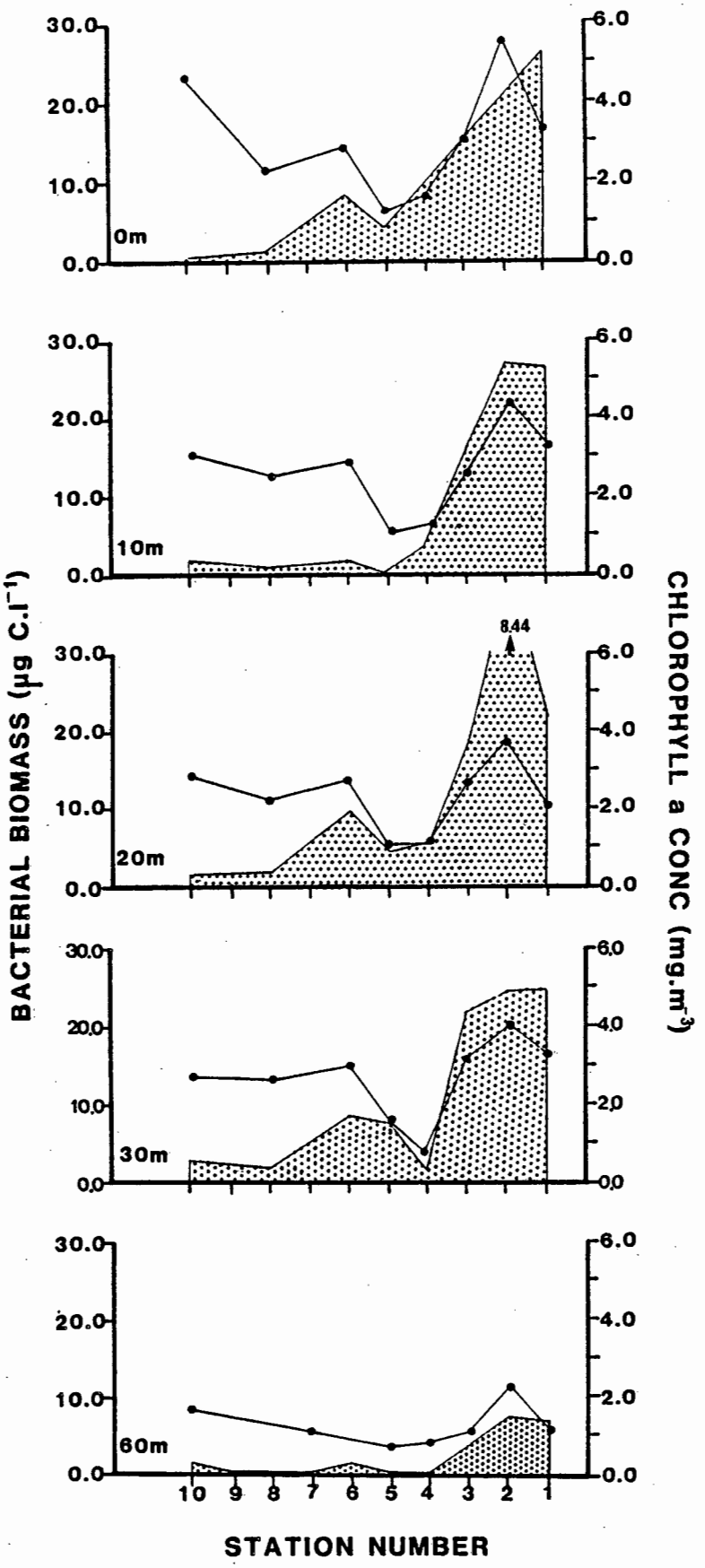

FIGURE 59: Horizontal distribution of bacterial biomass (—●— : µg $^{-1}$C.l^{-1}) and chlorophyll a (▒ : mg.m^{-3}) at specified depths: leg 3.

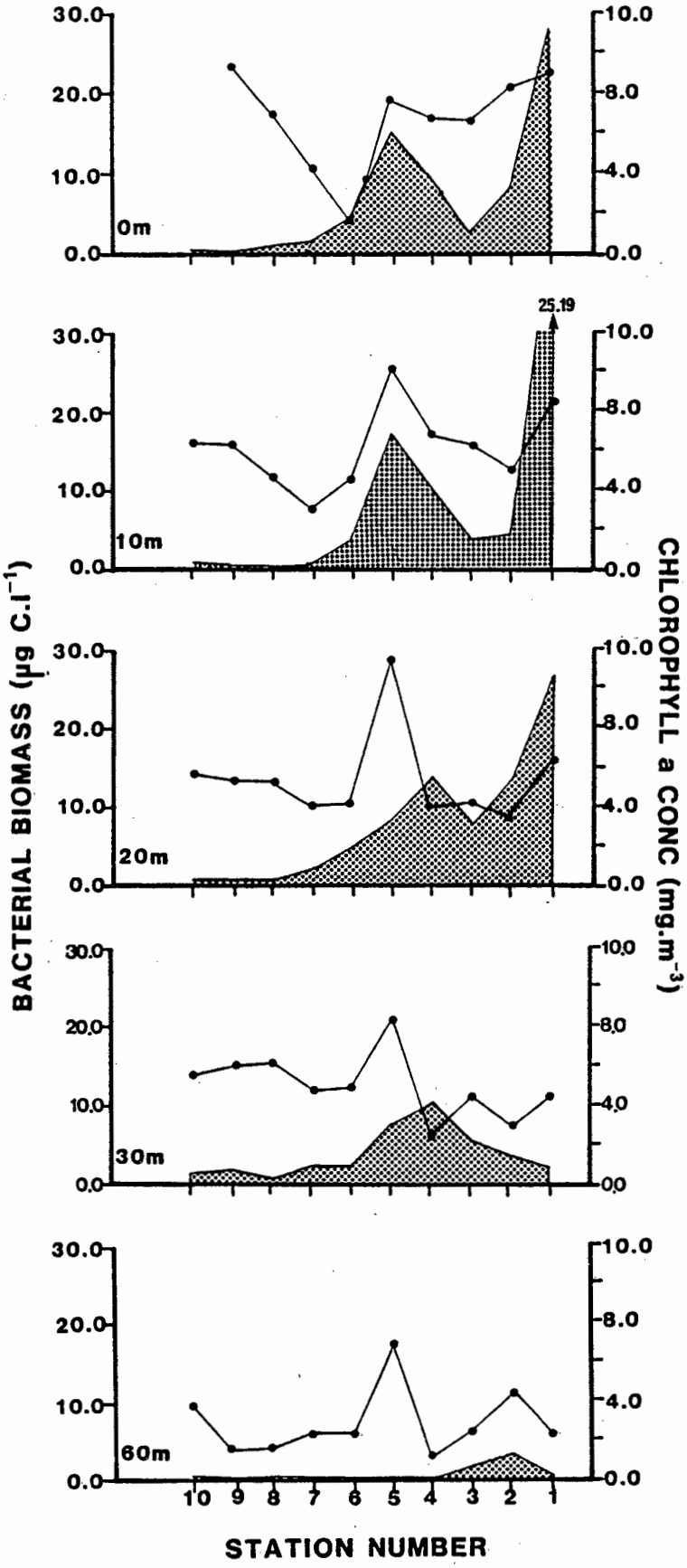

FIGURE 60: Horizontal distribution of bacterial biomass (—●— : µg $C.l^{-1}$) and chlorophyll a (▨ : $mg.m^{-3}$) at specified depths: leg 4.

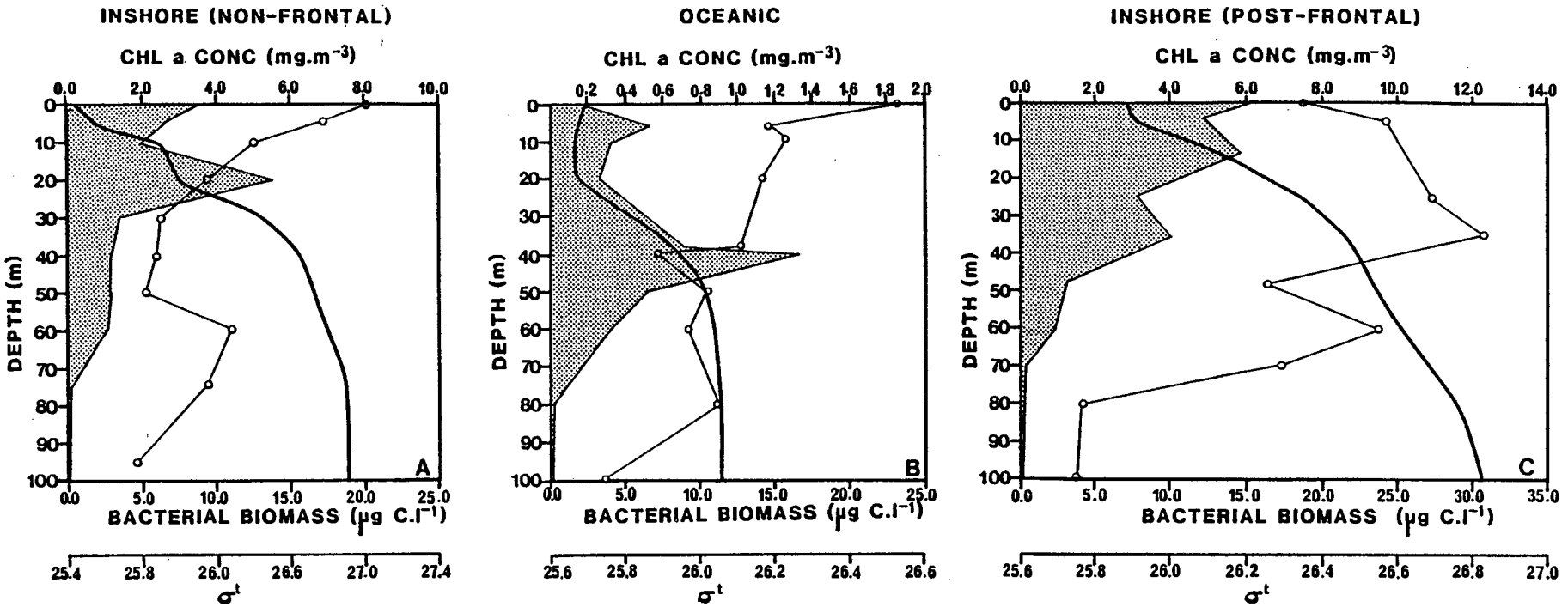

FIGURE 61: Vertical distribution of bacterial biomass (—o— : µg $C.l^{-1}$) and chlorophyll a (▩ : $mg.m^{-3}$) in relation to changes in density (—— : σ^t) at inshore, oceanic and post-frontal stations.

The tongue of oceanic water which interposed in the central waters (fig.49c) had a low bacterial content (numbers = 0.5 x $10^6.ml^{-1}$, biomass = 7.5µg $C.l^{-1}$) and the resultant formation of a thermal and σ^t front 17.79km offshore (figs 48c) resulted in the compression and deepening of the inshore bacterial bloom to 80m (fig.56b, 57b, 59), with the dispersion of the patch of high numbers and biomass previously noted. Within the front itself numbers and biomass were low, but the offshore bacterial distribution expanded behind the front, now extending to within 32km of the shore (fig.59).

The reformation of the front 29.00 - 35.00km offshore was accompanied by a marked change in the bacterial distributions. At the inshore face of the front a large patch of high bacterial numbers (2.0 x $10^6.ml^{-1}$) and biomass (20.0µg $C.l^{-1}$) formed, extending vertically to 60m depth. A secondary patch of increased numbers and biomass also formed at the inshore stations, probably in response to increased phytoplankton activity in this region. There was no marked drop in bacterial numbers and biomass at the front itself, although the horizontal distribution was still bimodal around the front, with onshore and offshore peaks on either side of the front (figs 56c, 57c, 60).

A variety of factors appeared to affect the distribution of bacteria. On each of legs 2,3 and 4, a strong correlation between bacterial abundance ($\mu g\ C.l^{-1}$) and Chl a concentration ($mg.m^{-3}$) was shown to occur (Table 9).

	n	a	b	r	p
leg 2	91	0.467	11.57	0.66	<0.01
leg 3	73	1.620	8.29	0.54	<0.01
leg 4	80	0.802	9.75	0.42	<0.01

Table 9: Values for the correlation $y = ax + b$, where
y = bacterial biomass (μg.bacterial $C.l^{-1}$)
x = Chl a concentration ($mg.m^{-3}$)
for legs 2, 3 and 4 of the cruise.

The position of the front (and hence physical factors) appeared to exert a strong influence on bacterial distributions and in addition it was noted that particle density distributions (figs 55a-c) bore a strong similarity to the overall bacterial distribution, particularly at stations inshore of the front.

4.3.3.11 Plateability

Bacterial plateabilities, as %P at specific depths (0m, 10m, 20m, 50m, 70m), are shown in figs 62a-e. In addition, the %P at six depths for 3 consecutive vertical profiles (before, during and after frontal development) at Station 3 (16.68km offshore) are presented in fig.63.

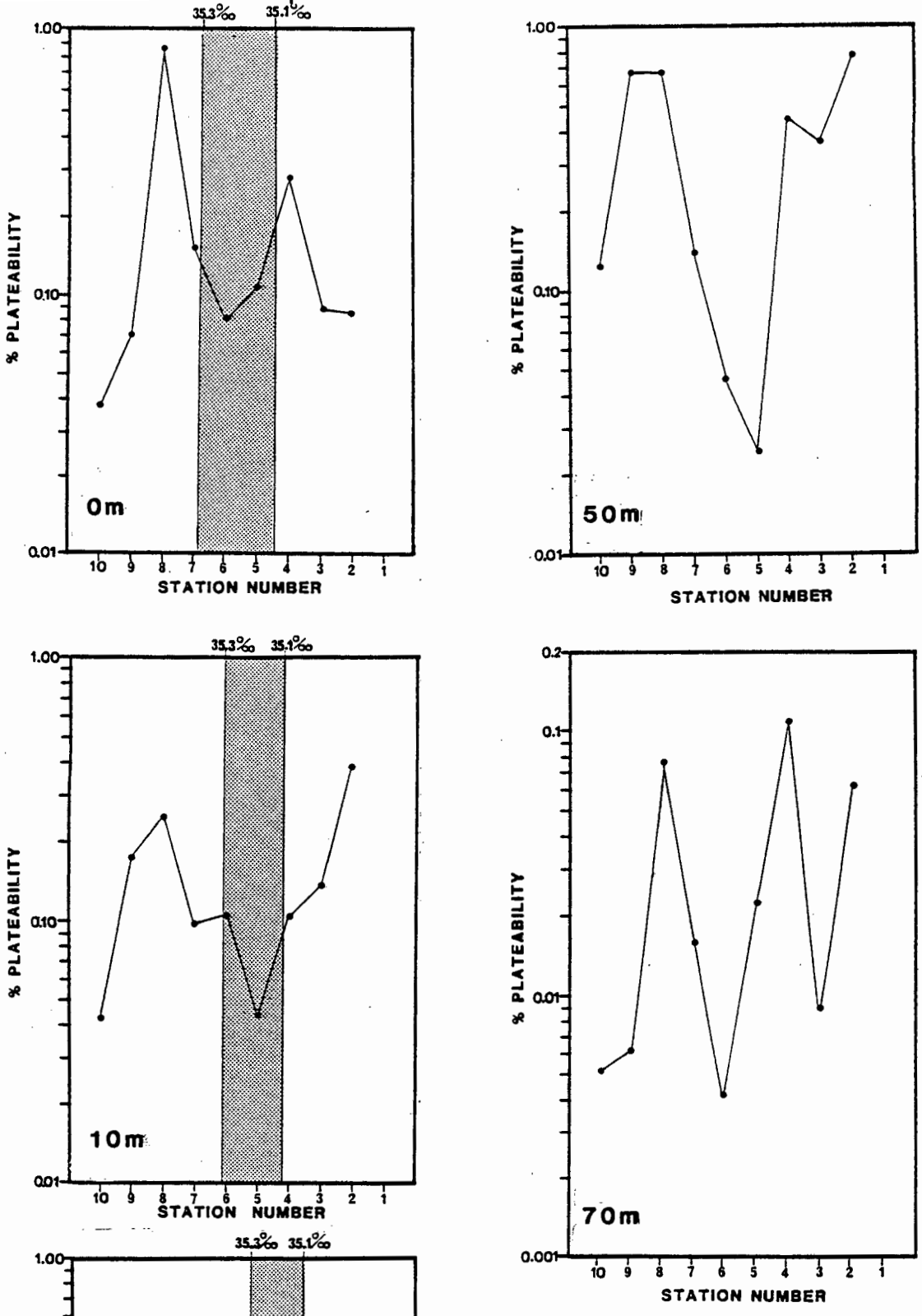

FIGURE 62: Changes in percentage plateability at specified depths with distance from the shore, assessed on 0.1% Pep-SWA, showing the frontal salinity boundaries (▒).

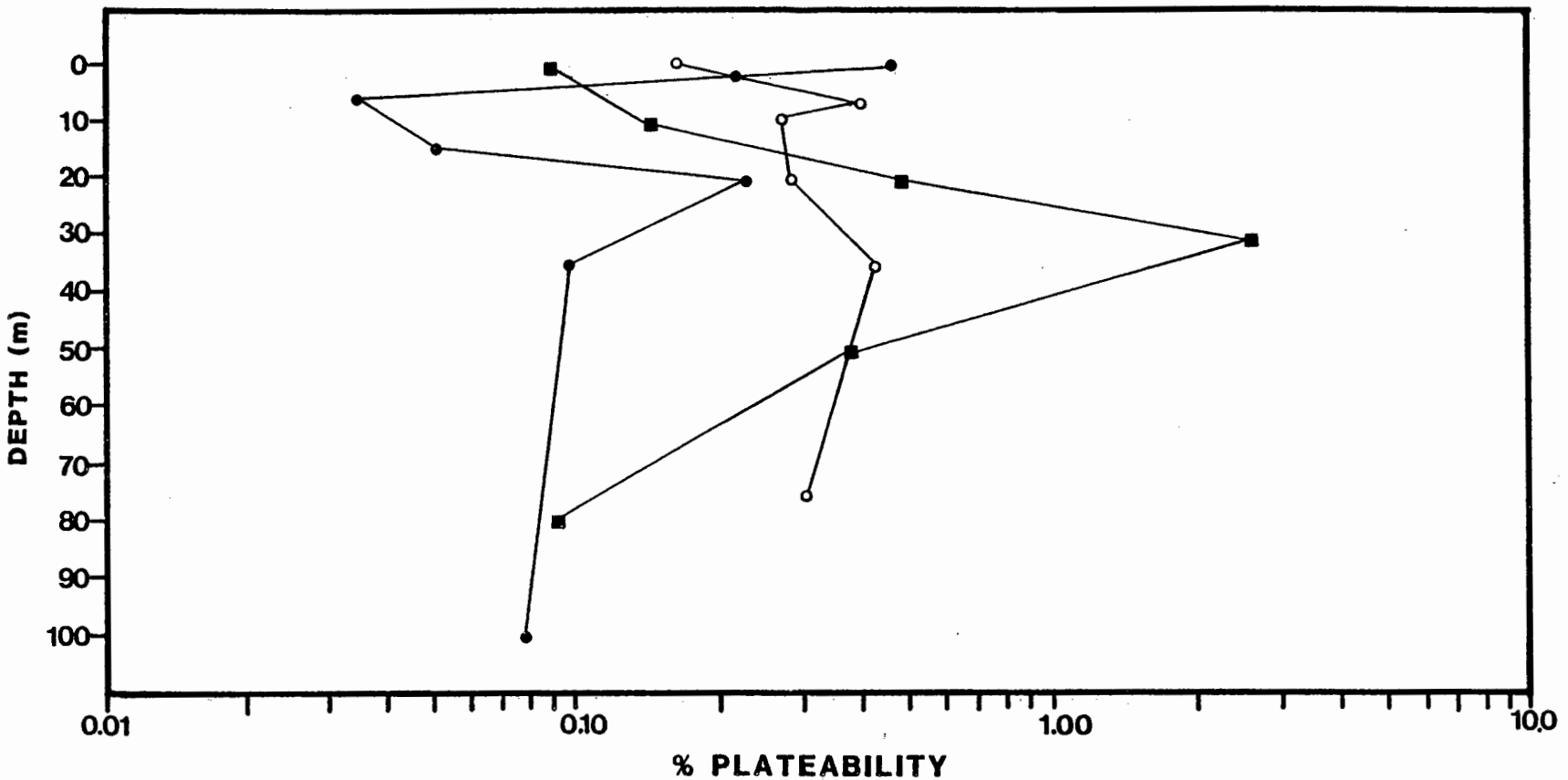

FIGURE 63: Changes in vertical distribution of percentage plateability at Station 3, before (leg 2), during (leg 3) and after frontal development (leg 4). (—●—:leg 2. —○—:leg 3. —■— :leg 4).

Horizontal distribution of percentage plateabilities was bimodal at all depths tested, with centres of high plateability at stations inshore of 22km and at stations between 45 and 50km offshore. The outermost station (55.60km) had very low plateabilities (0.100%) above the pycnocline although the sub-pycnocline plateability was slightly higher (0.125%). The stations above the pycnocline in the central waters (22 - 39km) showed plateabilities intermediate between the very low oceanic levels and the high levels recorded at stations on either side, while plateabilities below the pycnocline were very low (0.050%).

%P varied widely both horizontally and vertically and with time (fig.62, 63). In general, plateabilities were below 1% and in many cases below 0.1%. The vertical distribution appears strongly linked to the position of the pycnocline, with an increase in plateability from the surface to the pycnocline and a drop in plateability below it (fig.63).

Determinations of plateability at Station 3 before, during and after frontal development are shown in fig 63. Before development of the front (leg 2), plateabilities were highest in the surface waters, and fell to low level (<0.10 %) within 10m of the surface. A slight increase occurred at 25m, co-incident with the pycnocline, but sub-pycnocline plateabilities were also low. On development of the front (leg 3), the %P fell in the surface waters but increased at all other depths to >0.20 % . The variation in %P with depth was also markedly reduced. After passage of the front (leg 4), % P

above 20m and below the pycnocline fell once more, but continued to increase (to>1.0 %) on the pycnocline itself.

No correlation could be drawn between Chl a concentrations or nutrient status and the %P (all r values $<< 0.5$, $p>0.1$). In this instance, the area of low plateability in the central waters appears strongly linked to physical factors, since it is delineated by the physical factors defining the area of the front (fig.62a-c). Figs 62a-e show the disposition of the isohalines - the frontal area corresponds closely with the drop in plateability, but it should be noted that the same effect pertains below the pycnocline (figs 62d, e).

4.3.3.12 Heterotrophic uptake activity

Results of determinations of K^m, V^{max}, V^{max}/B and $^V/B$ are presented in figs 64 a - d.

In the oceanic waters at station 10, the K^m for glucose was low in both surface waters (1.59 $\mu g.l^{-1}$) and deep waters (0.83$\mu g.l^{-1}$). This suggests that high affinity uptake mechanisms predominate here as a response to perennially low ambient glucose concentrations. In the surface waters, the K^m for glutamic acid was also low (2.55$\mu g.l^{-1}$) but this rose to 4.66$\mu g.l^{-1}$ in the deeper water, suggesting that some source of amino acid occurs here.

At Station 3 the K^m values for both glucose and glutamic acid were higher than those at the offshore station at all depths, reflecting the higher ambient nutrient concentrations resulting from phytoplankton activity in this region. Before development of a front in

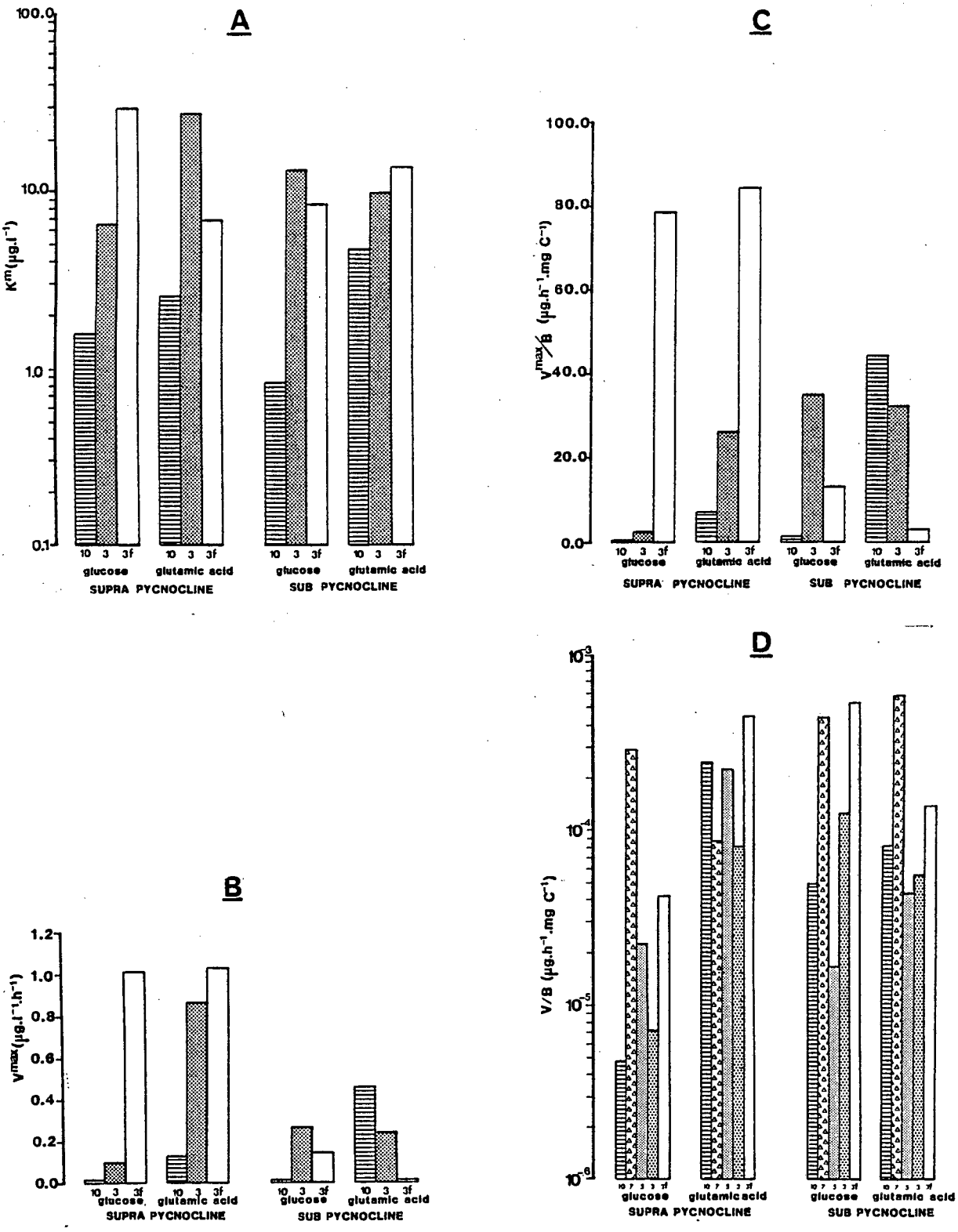

FIGURE 64: Changes in parameters of uptake of ^{14}C radio-labelled glucose and glutamic acid in relation to frontal structures. (Fig. 64a: K^m ($\mu g.l^{-1}$). Fig. 64b: V^{max} ($\mu g.l^{-1}.h^{-1}$). Fig. 64c: Specific heterotrophic potential (V^{max}/B = $\mu g.h^{-1}.mg\ C^{-1}$). Fig. 64d: Specific velocity of uptake of low concentrations of substrate (V/B = $\mu g.h^{-1}.mgC^{-1}$). : Station 10 (oceanic). : Station 7 (frontal). : Station 5 (frontal). : Station 3 (non-frontal). : Station 3 (frontal).

this region (fig.50b, 64b) the K^m for glucose in the surface water ($6.47\mu g.l^{-1}$) was lower than that of glutamic acid ($27.30\mu g.l^{-1}$). This is in keeping with the finding that an aged phytoplankton bloom was present in this region. On development of a thermal front in this region (fig.50b) this situation was reversed and the K^m for glucose was elevated above that of glutamic acid ($29.70\mu g.l^{-1}$ and $6.78\mu g.l^{-1}$ respectively). This suggests that the ambient glucose levels rose suddenly, probably as a result of rejuvenation of the phytoplankton bloom. In the deeper waters at this station the K^m values for glucose and glutamic acid remained similar before and after development of the front. V^{max} values are a reflection of the heterotrophic activity of the whole bacterial population. Overall, the V^{max} values for deep populations appeared lower than those of populations above the pycnocline (fig.64b).

In surface waters at the oceanic water station (55.56km) the V^{max} for both glucose ($9.49 \times 10^{-3}\mu g.\ell^{-1}.h^{-1}$) and glutamic acid ($0.14\mu g.\ell^{-1}.h^{-1}$) was low. In the deeper waters, however, the V^{max} for glutamic acid was higher ($0.49\mu g.\ell^{-1}.h^{-1}$) again suggesting the maintenance of competent amino acid uptake mechanisms by this population.

At Station 3, before development of the thermal front, the V^{max} value for both glucose ($0.10\mu g.l^{-1}.h^{-1}$) and glutamic acid ($0.87\mu g.l^{-1}.h^{-1}$) was low in the surface waters.

On development of a front in this region (figs.50b, 54b) a marked increase in the V^{max} for glucose (to $1.06\mu g.l^{-1}.\ h^{-1}$) and a lesser increase in the V^{max} for glutamic acid (to $1.14\mu g.l^{-1}.h$) was observed. These results suggest a fundamental change in the activity of the bacterial population in response to the development of the thermal front.

V^{max}/B (fig.62c) represents the heterotrophic potential per unit of bacterial biomass, a standardised measure of heterotrophic potential independent of population size (Hoppe, 1978). Similar patterns were shown to be those of the V^{max} statistics (fig.62b). At the oceanic station surface V^{max}/B measures were low for both glucose ($0.48\ \mu g.h^{-1}.mg\ C^{-1}$) and glutamic acid ($7.05\mu g.h^{-1}.mg\ C^{-1}$). In the deeper waters, however, although the V^{max}/B for glucose was still low ($1.45\mu g.h^{-1}.mg\ C^{-1}$), that for glutamic acid was fairly high ($44.70\mu g.mg\ C^{-1}.h^{-1}$), again reflecting the maintenance of amino acid uptake mechanisms by this

population. At the inshore station, the V^{max}/B ratio for both glucose (2.51µg.h^{-1}.mg C^{-1}) and glutamic acid (21.65µg.h^{-1}.mg C^{-1}) was low but reflected a higher active uptake of amino acids. With the development of the thermal front in this region, a very marked increase in the V^{max}/B occurred for both glucose (78.52µg. h^{-1}.mg C^{-1}) and glutamic acid (84.44µg.h^{-1}.mg C^{-1}). In the deep waters of this station, a drop in the V^{max}/B for both glucose and glutamic acid occurred with development of the front. Development of the front had led to an increase in bacterial biomass in the deeper waters (from 7.53µg $C.l^{-1}$ to 11.21µg $C.l^{-1}$) as the distribution of surface bacteria previously associated with an aged phytoplankton bloom (fig.55a, 57a) was distorted by the adjacent front (fig. 55b, 57b). This increase of biomass of inactive bacteria was responsible for the drop in presumably heterotrophic potential.

Patterns of uptake of low concentrations of dissolved organic substrates (glucose = 7nmol.l^{-1}, glutamic acid = 9nmol.l^{-1}) were complex (fig.64d). Two of the three frontal stations tested (Station 7 and Station 3, leg 2) showed markedly elevated uptake rates of glucose in the surface and deep waters, and of glutamic acid in the deep waters, compared with the offshore station and the inshore non-frontal station (Station 3).

Station 5, however, which was adjacent to the newly formed, weak front on leg 4, showed only a slight increase in uptake of glucose in the surface waters and otherwise the uptake patterns were similar to those of non-frontal stations. It is interesting to note that uptake mechanisms for glutamic acid were maintained by bacteria in the surface waters at Station 10 and Station 5.

A positive correlation existed between the bacterial plateability on 0.1% peptone agar and the low concentration uptake rates of glucose, as shown in Table 10.

	a	b	r	p
glucose	155.583	0.034	0.63	<.01>.005
glutamic acid	62.492	0.118	0.092	.1

Table 10: Values for the correlation equation
$Y = ax + b$ where Y = % Plateability
x = Specific uptake ($\mu g.h^{-1}.mg\ C^{-1}$)

4.3.4 Discussion

The four consecutive transects covered a period of 7 days, and showed a complex picture of an unstable and rapidly changing frontal system, produced as a result of the interaction of 4 water masses each with a characteristic salinity and temperature, modified by the additional effects of windstress and sunwarming. As a result, it was noted that thermal, salinity and density fronts were not always coincident (figs 48, 49, 50), but thermal effects appeared to exert the greatest influence on both σ^t profiles and the biological characteristics of the water column.

In the region sampled, upwelling of South Atlantic Central Water is more continuous than that further south, since it results from deflection of the northward flowing deep waters by the coast, although additional wind induced upwelling may also occur (Nelson and Hutchings, 1983). In this instance winds were predominantly of low velocity ($<10 km.h^{-1}$) so that active upwelling was limited. Two frontal systems were initially observed - the first dominated by the thermal effects resulting from confluence of the warm oceanic waters with cool inshore waters, and the second resulting from confluence of low salinity recently upwelled water with higher salinity aged upwelled waters (figs 48a, 49a).

Salinity is a more highly conserved characteristic than either temperature or density, and 4 major water masses were identified on this basis. Throughout the survey, a coastal body of low salinity (<35.0°/oo), recently upwelled water was maintained within 11.12 km of the coast. Oceanic water (salinity >35.4°/oo), was limited to distances greater than 40.0 km offshore. Between these two bodies of water was a large mass of mixed, aged upwelled water of intermediate salinity, while underlying these water masses, but contiguous with the recently upwelled water at the coast was the low salinity (<34.9°/oo) South Atlantic Central Water. A striking feature of this system was the spatial stability of the oceanic, coastal upwelled and South Atlantic Central Water masses. In the intermediate aged upwelled water, however, conditions were highly unstable and sunwarming, windstress and the movement into the area of patches of high salinity oceanic water led to the development and passage of thermal fronts which reflected as density (σ^t) fronts to a depth of at least 25 m.

On initial survey (figs 48a, 49a, 50a) the thermal, density and salinity fronts resulted from convergence of cold upwelled water with warm surface waters 22.24 - 38.92km offshore (Station 4-7). Rapid sunwarming of these surface waters caused the thermal and salinity fronts to separate (figs 48b, 49b) so that the thermal

front lay between 32km and 43km offshore, with a secondary salinity front lying between 19km and 25km offshore. The intrusion of a patch of high salinity oceanic water in the central waters, together with the shorewards movement of warm surface waters under the influence of onshore winds then caused the thermal front to move inshore yet again so that it lay between 18km and 25km, the closest inshore that it was observed to move. At this point, the distinct salinity front between recent and aged upwelled water had weakened and the σ^t profiles suggested that highly unstable mixed conditions prevailed above the thermocline inshore of the front (fig.50c). Further sunwarming, a relaxation of the winds (fig.47a, b) and the disappearance of the oceanic intrusion led to the offshore movement of the front again, so that it came to lie between 30km and 37km offshore. At this point the front was considerably weakened, but stratification was obvious at stations inshore of it (fig.50d) even in the surface waters.

From these results it was concluded that the transect was bounded by areas of relatively stable hydrological conditions, but that the central waters between 17km and 40km offshore were unstable and characterised by the frequent development and passage of temporary fronts, resulting in both horizontal and vertical mixing in this region.

Throughout the survey it was noted that passage of the front tended to elevate surface nutrient levels, particularly at the inshore face of the front. As a result, surface water patches of elevated PO_4 and NO_3 occurred at the front, as has been previously noted by Hutchings et al. (1985). The passage of fronts through the central waters appeared to maintain NO_3 levels above limiting concentrations (1µg at NO_3-N.l^{-1} [Barlow 1984]).

It was observed that areas with Chl a levels >1mg.m^{-3} were limited to waters of upwelled origin and that the distribution of Chl a remained very stable (fig.55). Initially, inshore of 18.0km, a large bloom was associated with the newly upwelled water. In the surface waters of this region, nutrient levels were very low, and the Chl a maximum lay near the pycnocline. This suggests that this bloom had already senesced and sunk (Eppley et al., 1967). With the inshore and offshore movement of the front, extensive mixing took place as is the thermocline and this bloom was mixed throughout the water column and rejuvenated, subsequently producing high Chl a levels (>20.0mg.m^{-3}) at the inshore stations by termination of the survey. Similarly, passage of the front through the central stations led to the development of a small bloom in the deeper waters at Station 6 which later extended

throughout the water column at station 5 and 4 (figs 58 - 60).

Passage of the front may also serve to mix waters (with their attendant biota) horizontally as well as vertically. Closely associated with the highly productive inshore area was a patch of high particle density (fig.54a) which initially appeared to be maintained close to the salinity front. This was subsequently deeply extended by downwelling at the thermal frontal face on its shoreward passage (fig.54b) in a very similar fashion to that described for Chl a levels by Hutchings et al. (1984, 1985). Thereafter, this patch was dispersed throughout the central waters when the front moved offshore once more (fig.54c). It can be concluded that while the overall Chl a distribution appeared stable, the presence and passage of fronts resulted in the development of localised areas of high phytoplankton production and subsequent dispersal of the cells. The distribution of phytoplankton is primarily determined by the presence of nutrient rich upwelled water in the euphotic zone, but as noted by Eppley et al. (1973), Eppley and Peterson (1979) and Legendre (1981), in the event of nutrient (and particularly inorganic N) losses from stratified suface waters by its incorporation into biomass (Barlow 1982a, b, 1984) and additional losses from the formation of

refractory particulates and the sinking of P.O.M. from the euphotic zone, maintainance of plankton blooms comes to depend upon renewal of nutrients across the pycnocline or frontal boundary. In addition to the increases in NO_3 and PO_4 associated with the frontal face, Hutchings et al. (1985) record that responsive increases in phytoplankton standing stock are a common feature of the Benguela frontal system, as was noted in this survey. Hutchings et al. (1984, 1985) showed that phytoplankton productivity increased at the frontal face although it remained low compared with inshore productivity, and suggested that in addition to stimulation of the bloom by nutrient augmentation, there is a mechanical accumulation of cells at the frontal face in response to limited upward water movement at the inshore face adjacent to downwelling in the convergent zone.

Probyn (1985) suggests that recycling and heterotrophic mineralisation may be enhanced at the front. Size fractionated nitrogen uptake studies showed that net plankton (>10μm) dominated both the biomass and nitrogen uptake in the inshore region, while offshore and at the front, nano- and picoplankton were more important. NO_3 uptake levels were enhanced at the front, however, suggesting an enhancement of microheterotrophic processes which may efficiently recycle low nitrogen

stocks, in addition to the enhancement of nutrient levels by mixing at the frontal face. Holligan et al.. (1984a, b) and Linley et al. (1983) showed that in stratified waters adjacent to a tidal mixing front, dinoflagellates were stimulated by mixing at the frontal face itself, and that increased heterotrophic activity was also present.

In contrast to the stability of Chl a distributions, however, the distribution and abundance of bacteria was observed to change rapidly, both horizontally and vertically. In general there was a drop in both numbers and biomass with depth (fig.61) with the pycnocline marking a disjunction in the distribution: numbers and biomass were much lower below the pycnocline. This effect has been noted elsewhere (Painting et al., 1985) and may be related to carbon deprivation below the pycnocline.

The highest bacterial numbers and biomass recorded occurred at distances inshore of 23.0km, and were clearly associated with waters of upwelled origin with high Chl a concentrations (Table 9). Numbers and biomass were however also high in far offshore waters (>40km) which is surprising, since Chl a levels there were low, and oceanic waters usually carry a low bacterial biota (Sorokin, 1971; Fuhrman et al., 1980; van Es and

Meyer-Reil, 1982). As previously discussed (Section 4.2) this may result from remote stimulation of bacterial populations in a manner similar to that described by Pingree et al. (1977), or by nutrient enhancement effects resulting from the abuttal of the oceanic and inshore waters (Savidge, 1976; Savidge and Foster, 1978). Unlike the population described in Section 4.2, however, this population was not associated with increases in the Chl a concentration in the offshore water (fig.41). In the central waters, and in water just offshore of the front, numbers and biomass of bacteria were low, so that horizontal distributions of both numbers and biomass had a bimodal form (figs 58 - 60). The distribution of bacterial biomass was strongly affected by the position of the front and in each case, the front marked the outer limit of distributions >10.0µg $C.l^{-1}$. As a result, movement of the front in and offshore resulted in changes in the distribution of biomass.

Increases in bacterial biomass at fronts have previously been noted by several authors: Fogg et al. (1985b), Lochte and Turley (1985), Egan and Floodgate (1985) and Turley and Lochte (1985) recorded increases in both numbers and biomass associated with frontal formation in the West Irish Sea, which they attributed to stimulation of the bacterial population by enhanced phytoplankton

activity as well as mechanical accumulation at the front itself. Holligan et al. (1984a, b) and Linley et al. (1983) noted pronounced increases in numbers and biomass at a front in the English Channel, largely attributable to nutrient stimulation by increased phytoplankton activity, while Hanson and Lowery (1985) noted similar increases aligned with Polar frontal zones, which they attributed to increased biological activity resulting from interaction of low nutrient Sub-antarctic waters with high nutrient Arctic waters, in a fashion similar to that described by Savidge (1976). The Benguela front is, however, distinctly different from other frontal systems in that it appears highly unstable. As a result bacterial numbers and biomass in the immediate vicinity of the front are reduced by vertical mixing of surface waters with low population density deeper waters, with increases in numbers and biomass occurring on either side but remote from the front itself. A similar effect was noted with particle distributions (fig.54), suggesting that mechanical dispersion and accumulations occur with frontal development and movement, but that there was an additional association between phytoplankton growth and increases in bacterial numbers and biomass.

Bacterial blooms were seen to occur in association with

increased Chl a concentrations at inshore stations (fig. 58 - 60) and were also seen to follow the development of phytoplankton blooms at the central and coastal stations following passage of the front and rejuvenation of blooms. A similar effect was noted by Jacobsen et al. (1985) following an intrusion of nutrient rich Gulf Stream water into nutrient depleted coastal waters off Florida, and by Turley (1985) and Fogg et al. (1985b) in the West Irish Sea.

It can be concluded that multiple factors affect bacterial numbers and biomass distribution, and their interaction may be complex: in addition to a close association with phytoplankton distributions, bacterial distributions are also affected by particle distributions, water movement and frontal phenomena to a far greater degree than the phytoplankton. In this instance the centre of high bacterial biomass and numbers inshore was compressed and expanded by frontal movement and low biomass occurred behind the front as a result of advection to the surface of low population density waters.

At all depths tested, bacterial plateability (figs 62a-e) showed a bimodal distribution. Highest %P was recorded at the inshore stations where active phytoplankton bloom development was taking place and adjacent to the frontal zone in the offshore waters, while %P in the frontal zone

itself was low. As discussed in Section 3, the factors affecting bacterial plateability are complex and increases in plateability arise during phases of active uptake of dissolved organic carbon (such as occurs during active phytoplankton bloom development), during mineralisation of refractory particulates and during fragmentation in response to the onset of starvation. In this instance a strong correlation was found between velocities of uptake of low concentrations ($<10 nmol.l^{-1}$) of glucose and the %P (Table 10) suggesting that, for the stations inshore of the front, changes in plateability do reflect differences in heterotrophic metabolic activity. The increase in bacterial plateability at stations further offshore than this are however unassociated with increased phytoplankton activity, but it will be noted that at the time that the plateabilities were assessed, a considerable increase in the amount of particulate material occurred in this area due to the dispersal of the inshore particle patches by offshore movement of the front, which may have been responsible for stimulation of the highly oligotrophic offshore populations. The low plateabilities in and immediately adjacent to the front itself probably arise due to advection to the surface of nutritionally incompetent cells from deeper waters. After passage of the front, development of phytoplankton blooms and resulting exudation of D.O.C. results in increases in plateability.

This was exemplified by three consecutive platings at Station 3 (16.67km) before, during and after passage of the front (fig.17). Plateabilities at all depths were low before development of the front here, since this was an area occupied by an aged phytoplankton bloom. Development of the front restimulated the bloom and an immediate increase in plateability occurred. After passage of the front, this bloom continued, particularly at the pycnocline, but the surface portion of the bloom was dispersed behind the front and a corresponding decrease in plateability in the surface waters was noted while plateabilities continued to rise between 20m and 30m. It should be noted that this is the result of two effects: stimulation of the phytoplankton bloom by nutritional enhancement and mixing to optimum light levels, and mechanical dispersal of cells horizontally by movement of the front. In addition the effect of movement of the front through a senescing bloom would appear to produce a different effect to its movement through an area of low Chl a concentration. In the first case there appears to be an immediate rejuvenation of both phytoplankton and bacterial cells. In the second case, plateabilities at the front itself were lowered, but rose after passage of the front and subsequent phytoplankton development. It should further be noted that there is a time lag between maximum phytoplankton

development and maximum bacterial plateability (Section 3). This is apparent in the case of plateabilities of bacteria associated with bloom development at Station 6, 5, and 4 where phytoplankton blooms were too young for any marked increase in plateability to have occurred, although increases in numbers and biomass had occurred. Such a lag was previously noted under experimental conditions (Section 3).

Although there was a marked delay between onset of phytoplankton growth and bacterial plateability increases, the delay in development of mechanisms competent to take up low concentrations of D.O.C. was much shorter and these provided a better measure of heterotrophic activity (Hoppe, 1977; Vaccaro and Jannasch, 1966; Lochte, 1985).

During this survey, it was found that in offshore waters, where Chl a, levels were low, only high affinity uptake mechanisms were maintained for glucose, although a low affinity uptake mechanism for glutamic acid appeared to predominate in the waters below the pycnocline. It was felt that this was a reflection of the low ambient dissolved organic carbon concentrations and the dependence of the bacterioplankton on particulate and probably refractory organic matter.

At the inshore stations, the development of the front clearly had an effect on the heterotrophic uptake activity, reflected in a rise in the K^m, V^{max} and specific heterotrophic potential for both glucose and glutamic acid (fig.64a-c) in the waters above the pycnocline and for glucose in the deeper waters, reflecting the dependence of these populations on dissolved organic substrates and hence a correlation between their level of activity and the physiological state of the phytoplankton blooms with which they were associated. Specific velocities of the uptake of low concentration ($7nmol.l^{-1}$) of glucose were shown to be higher at stations close to the front (fig.64d) than those at offshore stations or nonfrontal inshore stations, although glutamic acid uptake velocities remained high even in surface waters at the offshore stations.

The influence of ambient D.O.C. levels has to be inferred in this instance, where they were not directly measured, but clear assocations between increased rates of specific uptake and V^{max} and decreases in K^m have been shown to be associated with increases in ambient D.O.C. levels by Lochte (1985), Davis and Robb (1985) and in the present work (Section 3).

Throughout this survey, frontal development appeared to effect mixing of nutrient rich waters from below the pycnocline with impoverished aged upwelled waters above it. This appeared to stimulate rejuvenation of phytoplankton blooms, with associated increases in bacterial numbers and activity. In addition, bacterial populations in offshore waters appeared to be stimulated to a certain extent by the proximity of relatively rich inshore waters; the frontal zone was thus bounded by areas of relatively high bacterial biomass, with centres of high heterotrophic activity limited to the surface waters inshore of the front, and to areas of active phytoplankton growth.

SECTION 5: SUMMARY AND CONCLUSIONS

Upwelling areas, such as the Benguela region off the west coast of South Africa, are characterised by the regular advection to the euphotic zone of nutrient rich bottom water, during upwelling events which are usually wind induced. In the course of this study, it was shown that upwelling events occurred throughout the year, although they were most common during summer.

During summer, upwelling events occurred with a marked regularity with approximately 10 days elapsing between the height of upwellings. During winter, however, inter-upwelling periods were much longer. Whether they ocurred in summer or winter, upwelling events resulted in marked drops in water temperature, and increases in the nutrient content of the surface waters, as has been previously discussed by Andrews and Hutchings (1980) and Shannon (1985b).

South Atlantic Central Water, which is brought to the surface during such upwelling episodes, is very old, with a bottom residence time >500y (Morita, 1984; Shannon, 1985b) and has a very low D.O.C. and P.O.C. concentration. The bacterial populations associated with it have therefore been subjected to starvation conditions for a long time, and upwelling events can be regarded as effecting a rapid change in environmental conditions on such populations as this water is brought up to the euphotic zone.

The populations of plateable bacteria associated with upwelling water appear to be characteristic: whether upwelling occurred in winter or summer, the associated plateable populations were highly diverse, dominated by facultative anaerobic isolates and low in biomass.

As shown by Barlow (1982b, 1984) upwelled water is rapidly colonised by phytoplankton, which bloom within 5 days of an upwelling event, producing high Chl a concentrations in the nearshore region and for some distance offshore. These blooms result in the impoverishment of nutrients, particularly N and P, after which the blooms decline and senesce. Such phytoplankton blooms produce large quantities of D.O.C. and P.O.C. (Ignatiades and Fogg, 1973; Lancelot, 1984) which are utilised by bacteria (Lancelot, 1979; Larsson and Hagstrom, 1979, 1982).

In these studies, the bacterial populations associated with upwelled waters were shown to bloom in concert with phytoplankton populations within 5 days of exposure to a high light regime, with bacterial population development lagging behind phytoplankton bloom development by 1-2 days. The development of phytoplankton blooms, which produce a gradual increment of available nutrient, is very similar to the conditions described by MacDonell and Hood (1982) as being necessary for the revival of dormant,

starved strains. Associated with the development of the bacterial populations, changes in the structure of the populations of plateable bacteria were shown to occur: the diverse population of upwelled water was replaced by a low diversity population dominated by oxidative strains with a wide variety of catabolic properties. This population was also extremely active in taking up D.O.C. supplied in the form of ^{14}C radiolabelled substrates, and biomass specific uptake rates were shown to increase as phytoplankton abundance, D.O.C. and P.O.C. levels rose.

The blooming of phytoplankton cells was shown to deplete the nutrients in the mesocosm enclosure, as discussed by Barlow (1984). As the phytoplankton bloom declined, a decrease in the D.O.C. concentration occurred, and deprived of this source of nutrient, bacterial populations rapidly utilised the available P.O.C., and then themselves declined as the onset of starvation conditions and flagellate predation reduced the population. During this population decline, the diversity of the population increased, and facultative anaerobes once again appeared in the plateable assemblages. It was hypothesised that a similar sequence of events takes place associated with natural phytoplankton blooms. Continuous (daily) sampling at an upwelling site however, showed that during summer, the regularity of upwelling and the short inter-upwelling periods continually renewed the nutrient stocks in the

nearshore region, maintaining both phytoplankton and bacterial populations in an early stage of development - during summer, little difference between upwelling and downwelling plateable populations could be discerned. All summer populations tested were characteristically diverse with a high proportion of facultative anaerobes, with only slight decreases in diversity being noted after lengthy upwelling-downwelling cycles. During winter, however, a stationary phase population of low diversity, dominated by obligate aerobic isolates arose during the lengthy inter-upwelling periods. Similarity analysis of the plateable populations of summer, winter and the mesocosm enclosure (Fig.65) shows that the winter terminal population was most similar to the initial stationary phase population occurring in the mesocosm.

Sampling at such a fixed station, however, results in the repeated sampling of waters moving on and offshore in a fairly restricted scale. Hutchings (1981) and Hutchings et al. (1984, 1985) suggest that upwelled water may be exported from the shore in an approximately north-westerly direction for some distance. This water may be returned to the shore during downwelling, but it also mixes with offshore waters, and the hydrological characteristics of the region inshore of the front were shown in this study to be largely determined by the upwelled origin of these waters. Transects across the region from the shore to the

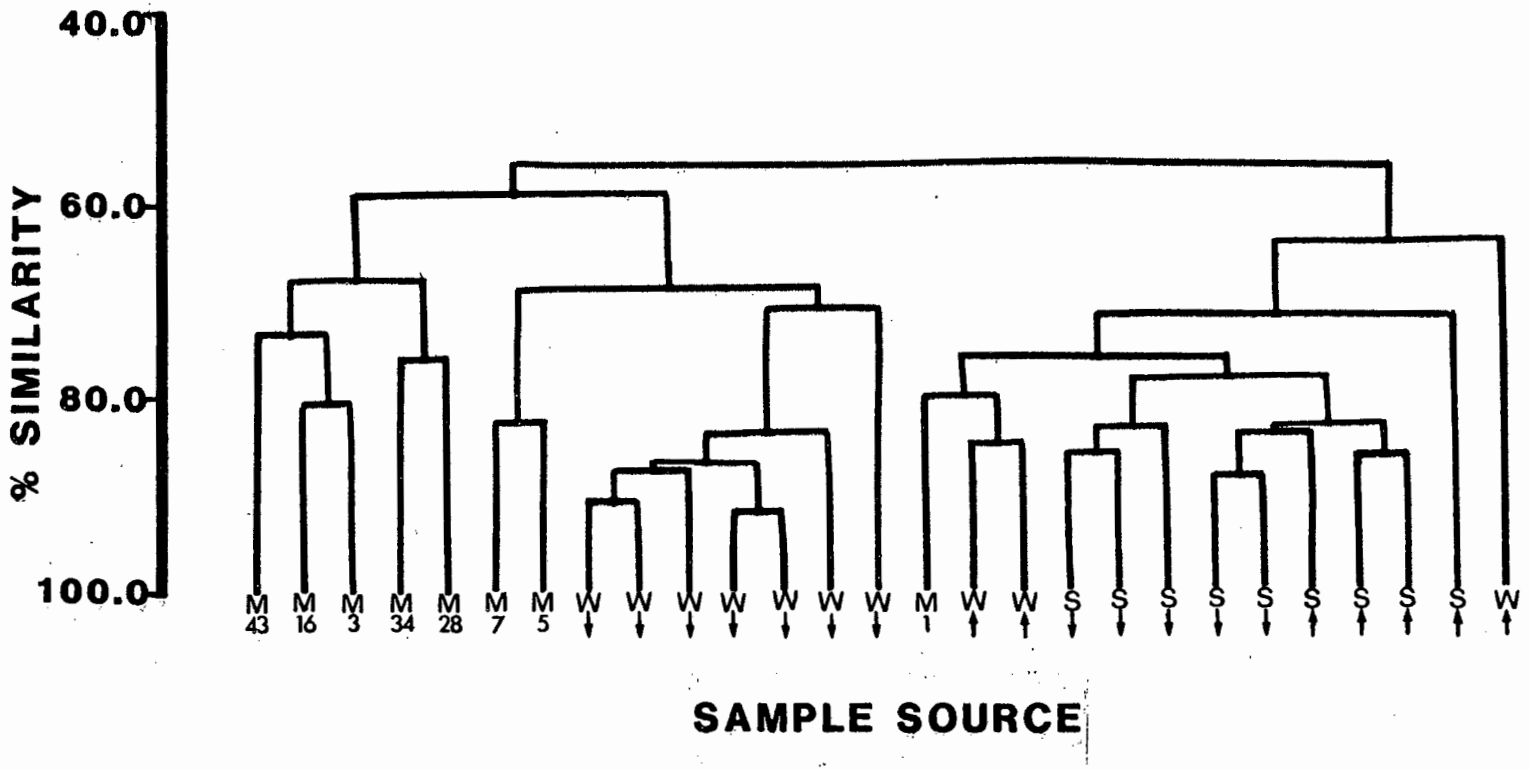

FIGURE 65: Results of similarity analysis of all randomly selected populations. (M = mesocosm enclosure (Section 3). W = winter sampling period. S = summer sampling period (Section 2). ↑ = days of upwelling. ↓ = days of downwelling.)

oceanic front confirmed that a drop in N and P occurred from the inshore region to the offshore region in the surface waters, and associated with this, a drop in the Chl a concentration, P.O.M. and numbers and biomass of bacteria also occurred.

Within this region, however, the development and passage of fronts was shown to exert a profound influence on bacterial activity. The passage of such fronts effected deep mixing of surface and deep waters across the pycnocline. While such mixing did not produce surface nutrient levels as high as those which occurred in upwelled water, it appeared to maintain localised nutrient levels above limiting levels, which was sufficient to stimulate the rapid development or rejuvenation of offshore phytoplankton blooms. In addition, these fronts appeared to produce some mechanical accumulation of both phytoplankton and bacterial cells, and may also have been responsible for maintaining phytoplankton cells in an optimum light regime. In proximity to these fronts, parameters of heterotrophic uptake of glucose in particular were shown to increase markedly, probably in response to increased D.O.C. release by reactivated phytoplankton blooms. Biomass specific uptake parameters (V^{max}/B, V/B) increased markedly after frontal development, and were comparable with those recorded at the height of the phytoplankton bloom in the mesocosm

enclosure experiment. An interesting feature was the close correlation between bacterial plateability and biomass specific uptake rates of low (<10 $nmol.l^{-1}$) concentrations of glucose, which confirmed the suggestion of Laake et al. (1983b) that plateability may serve as an indicator of nutritional capability and metabolic activity of bacterial populations.

A seemingly paradoxical feature of transects of the Benguela region however was the bimodal distribution of bacterial biomass and plateability across the oceanic front. On the front itself, mixing of low biomass deep waters with the high biomass surface waters produced an overall decrease in bacterial biomass. Offshore of the front however, distinct peaks in biomass were noted in oceanic waters, associated with which were marked increases in plateability, but not in the uptake of D.O.C. Such increases in biomass were unsupported by increases in Chl a, and all other measured nutrients were low. The plateable population of oceanic bacteria was shown to be typical of a bacterial bloom, being low in diversity and dominated by oxidative isolates. This suggests that the front marks the juxtaposition of two different bacterial populations, the inshore population being dependent on relatively higher available nutrients, and the oceanic population being adapted to highly oligotrophic conditions. In this instance, the oceanic

populations appeared to respond to the slight increases in nutrients, and particularly P.O.M. which occur at the front.

In the case of bacterial populations monitored during the mesocosm enclosure, the initial population reached very low levels after 10 days, following the senescence of the phytoplankton bloom. Following this, however, a second population of bacteria arose, which appeared to be dependent on recalcitrant D.O.C. and P.O.C. remaining in the mesocosm. This population was similar in generic structure to the initial population but quite different in properties, showing limited catabolic activity and failing to take up ^{14}C radiolabelled substrates.

The development and decline of these populations can be regarded as a temporal sequence which could equally well be applied to field phenomena, such as the sedimentation of particles through the water column or the export and mixing of waters from the inshore area. Itturiaga (1979) suggests, for example, that during sedimentation of particles, the small molecular weight compounds are utilised first and the larger molecular weight compounds last. It might be expected, therefore, that bacterial populations comparable to the first noted in the mesocosm would be associated with particles close to their point of origin, while populations corresponding to the second

stage of development in the mesocosm would only be found at the pycnocline or in deeper waters. Similarly, since extensive phytoplankton activity is limited to the inshore areas of the Benguela System, the export of these waters towards the front may occasion a similar change in the properties of the associated phytoplankton and bacterial populations. By the time waters approach the front, they may carry only recalcitrant D.O.C. and P.O.M. which are then available for utilisation by highly oligotrophically adapted oceanic bacteria.

This work has demonstrated the close interaction between phytoplankton bloom development and the development and activity of bacterial populations in the Benguela region. Bacterial populations in the field and in laboratory experiments were shown to respond rapidly to changes in physiological state of phytoplankton blooms, passing through a well-ordered and characteristic series of successions similar to those described by other authors in both fresh water and marine systems (Jones, 1973; Martin and Bianchi,1980; Hauxhurst et al., 1981; Fukami et al., 1985a, b).

While previous work has suggested that marine heterotrophic bacteria play a vital role in remineralisation and recycling processes, that of Lucas, (1985) and Ducklow et

al. (1986) suggests that these bacteria may in fact act as a carbon sink in the system: this is a vital question which remains to be answered in the Benguela Upwelling System where rapid recycling of nutrients is essential to maintain high productivity (Probyn, 1985). The results of this work however suggest that any further research must take the population structure of the bacterial assemblages into account, since it is essential to understand the physiological properties of the active populations before valid conclusions on their ecological role can be made.

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APPENDIX

Media and reagents

Catalase reagent

2.0% (v/v) H_2O_2 in distilled water.

(Cowan and Steel, 1970)

Carboxymethylcellulose (CMC) plates

Low viscosity CMC	10.0g
Aq. Dist.	100.0mℓ
Filtered seawater	900.0mℓ
Bacto-peptone	1.0g
Yeast extract	0.1g
Fe_2SO_4	0.01g

Zones of activity are visualised by flooding the plate with a 0.1% w/v (aq.) solution of Congo Red for 15m. Stain is then fixed with a 0.1 N HCl solution.

(Modified from Teather and Wood, 1982)

Frazier's reagent (for visualisation of gelatin liquefaction)

Mercuric oxide	12.0g
Aq. Dist.	80.0mℓ
HCl (conc.)	16.0mℓ

(Cowan and Steel, 1970)

Gelatin-Seawater Agar

Gelatin	10.0g
0.5% Peptone-seawater agar	1000.0mℓ

(Modified from Cowan and Steel, 1970)

Gran's reagent (for visualisation of agrase activity)

0.05m iodine in 0.12m KI.

(Hodgson and Chater, 1981)

Griesler's reagents (to show presence of NO_2)

Solution A:	Sulphanilic acid	0.8g
	5N Acetic acid	100.0mℓ
Solution B:	dimethyl-a-napththylamine	0.6g
	5N Acetic acid	100.0mℓ

(Cowan and Steel, 1970)

Oxidase reagent

tetramethyl-p-phenylenediamine	1.0g
Aq. dist.	100.0mℓ
Ascorbic acid	0.001g

(Cowan and Steel, 1970)

Seawater-nitrate broth

0.1% Peptone-seawater broth	1000.0mℓ
KNO_3	1.0g

(Modified from Cowan and Steel, 1970)

0.1% Peptone-seawater broth

Bacto-peptone	1.0g
Yeast extract	0.1g
Fe_2SO_4	0.01g
Aq. Dist.	250.0mℓ
Seawater	750.0mℓ

0.5% Peptone-seawater broth

Bacto-peptone	5.0g
Yeast extract	1.0g
Fe_2SO_4	0.01g
Aq. Dist.	250.0mℓ
Seawater	750.0mℓ

0.1% Peptone-seawater Agar

0.1% Peptone-seawater broth	1000.0mℓ
Agar	15.0g

0.5% Peptone-seawater Agar

0.5% Peptone-seawater broth	1000.0mℓ
Agar	15.0g

Seawater Cetrimid agar

Cetrimid-agar (Merck)	45.5g
glycerol	10.0mℓ
Aq. dist.	200.0mℓ
Seawater	800.0mℓ

Seawater skim-milk agar

Skim milk powder	10.0g
Aq. dist.	100.0mℓ
0.1% Peptone-Seawater agar	800.0mℓ

(Kaneko et al., 1979)

Seawater Peptone-glucose broth

0.1% Peptone-seawater broth	1000.0mℓ
glucose	5.0g

Seawater Peptone-mannitol broth

0.1% Peptone-seawater broth	1000.0mℓ
mannitol	5.0g

Thiosulphate-Citrate-Bile Salt agar plates (T.C.B.S.)

NaCl	10.0g
Sucrose	20.0g
Sodium citrate	10.0g
Sodium thiosulphate	10.0g
Peptone	10.0g

Ox bile	5.0g
Yeast extract	5.0g
Sodium taurocholate	3.0g
Ferric citrate	1.0g
Bromothymol blue	0.04g
Thymol blue	0.04g
Agar	15.0g pH = 8.6

(Kobayashi et al., 1963)